Cancer Treatment Reports

A Publication of the National Cancer Institute

Volume 70
Number 10
October 1986

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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1249 ANNOUNCEMENTS

Automated Fluorescent Analysis for Drug-Induced Cytotoxicity Assays[1,2]

Keiko Funa,[3,*] Nancy Dawson,[4] Phillip B. Jewett,[3] Hans Agren,[5] John C. Ruckdeschel,[6] Paul A. Bunn, Jr,[7] and Adi F. Gazdar[3]

The human tumor clonogenic assay has been reported to predict for sensitivity of human tumors to a variety of drugs. However, this assay requires large numbers of viable cells, is time-consuming, and takes at least 2 weeks before results are available. To circumvent these problems, Weisenthal developed a microscope-based dye exclusion assay. Because this method is also time-consuming and subject to observer error, we have developed an automated method of quantitating drug cytotoxicity using a flow cytometric cell sorter (FCM). After incubation of drug-exposed tumor cells, acetaldehyde-fixed duck red blood cells (DRBC) are added. Dead tumor cells and the fixed DRBC are stained by the fluorescent dye propidium iodide, which penetrates dead cell membranes. A two-parameter analysis (cell size as measured by narrow angle light scatter vs propidium iodide fluorescence) enables determination of the live tumor cell:DRBC ratio. There was a strong correlation between the FCM method and manual counting ($r = 0.958$ for cell lines, $r = 0.831$ for fresh leukemic cells, $P < 0.0001$ in both cases). We conclude that the automatized FCM method gives compatible results to the manual dye exclusion assay and increases efficiency. [Cancer Treat Rep 70:1147–1151, 1986]

In vitro assays for the preclinical screening of potential antineoplastic drugs and the selection of the "best" individual chemotherapy regimen may have a major impact in attaining improved cancer therapies. Recently, dye exclusion assays were re-evaluated following Weisenthal's (1,2) introduction of a modified assay based on calculating the ratio between living tumor cells and duck red blood cells (DRBC) added as an internal control. This assay has several advantages over the widely utilized agar cloning assay (3): (*a*) the assay can be used for tumor cells that do not clone in agar; (*b*) fewer cells are needed; (*c*) results are obtained rapidly; and (*d*) tumor cells can be distinguished from stromal cells. A preliminary comparison of these techniques has shown that their results are comparable (2). However, the dye exclusion assay utilizes microscopic evaluation and enumeration of the number of living tumor cells. This is tedious, time-consuming, and subject to observer error. In an effort to overcome these problems, we have developed a flow cytometric cell sorter (FCM) method to determine the ratio of live cells to DRBC. A direct comparison between these methods showed a highly significant correlation ($P = 0.0001$).

MATERIALS AND METHODS

Cell Lines and Tumor Cell Preparation

Three human cell lines, a promyelocytic leukemia cell line, HL60 (4), a small cell lung cancer (SCLC) line, NCI-H209 (5), and a non-small cell lung cancer (NSCLC) line, NCI-H460 (5), were maintained in continuous culture in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (GIBCO). Fresh leukemic cells from a 24-year-old male with relapsed acute T-cell lymphocytic leukemia, a 19-year-old male with newly diagnosed myelomonocytic leukemia, and a 62-year-old male with acute myelogenous leukemia of recent onset were obtained from peripheral blood and/or bone marrow specimens. Bone marrow cells (BMC) contained 90%–100% blast cells, whereas only 80% of peripheral blood cells (PBC) were blasts. Leukemic cells were isolated by Ficoll-Hypaque density gradient cen-

[1] Received Oct 15, 1985; revised May 21, 1986; accepted June 3, 1986.

[2] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Department of Defense.

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trifugation, washed twice with growth media, and counted. The viability of the tumor cells as determined by trypan blue dye exclusion was > 90% in all cases.

Disaggregation of Cells

Cells growing in aggregates, such as SCLC, have to be dispersed before FCM analysis, since only single cells can be counted. Various methods for dispersion were examined. Chelating agents (EDTA; Sigma Chemicals, St. Louis, MO) were not efficient; mechanical disaggregation (repeated pipetting using spinal needles) resulted in lower viability of cells; and ionic detergent (Zwettergent; Calbiochem-Behring, La Jolla, CA) was difficult to use in appropriate concentration and time. Enzymatic dispersion was superior to other methods for efficiency and cell viability. Thus, the floating SCLC cell line NCI-H209 and the adherent non-SCLC line NCI-H460 after 4 days of incubation were resuspended in 1 ml of enzymatic solution of 0.05% collagenase (CLSPA; Worthington Diagnostic System, Freehold, NJ), 0.1% trypsin (Sigma), and 2% bovine serum albumin (Sigma) in phosphate-buffered saline (PBS) and rotated in a test tube rotator at 37°C for 30 mins. Bovine serum albumin was used instead of fetal bovine serum in order to avoid the antitrypsin activity of the latter. Chicken serum also lacks antitrypsin activity and may be substituted for albumin (6). Then 0.1 ml of 1% soybean trypsin inhibitor (Sigma) in PBS was added immediately and washed by addition of 3 ml of PBS. Hyaluronidase and elastase (Worthington) were also tested in combination with other enzymes, but did not result in increased efficiency. Disaggregation was not required for the single-cell suspension leukemic cells.

Drugs

All drugs were obtained from the Division of Cancer Treatment, National Cancer Institute. They were diluted to a concentration 100 times the desired final concentration in PBS and stored at −70°C before use. The cell lines were exposed to etoposide and doxorubicin for 1 hr only. The fresh leukemic cells were exposed to the following drugs. Concentrations of drugs were used in three dilutions (0.1, one, and ten times the numbers given below; first number for 1-hr exposure and second for continuous exposure). The drugs were amsacrine (50, 0.75 μg/ml), cytarabine (100, 1.0 μg/ml), L-asparaginase (0.5, 0.05 μg/ml), 5-azacitidine (2.5, 0.084 μg/ml), dexamethasone (4, 0.4 μg/ml), daunorubicin (5, 0.5 μg/ml), mitoxantrone (—, 0.1 μg/ml), methotrexate (—, 1.0 μg/ml), mechlorethamine (3.5, — μg/ml), vincristine (0.8, 0.08 μg/ml), etoposide (125, 1.25 μg/ml), and doxorubicin (1.2, — μg/ml).

Dye Exclusion Assay

The assays were performed according to Weisenthal et al (2). Briefly, 0.1 ml of tenfold concentrated drugs was aliquoted into 4-ml polypropylene tubes and the desired number of tumor cells, 5×10^4 to 1.4×10^5 for the manual method and $1\text{–}2 \times 10^5$ for FCM, suspended in 0.9 ml of media, was added to the tubes. The cells were exposed to drugs for 1 hr or continuously. In control tubes, PBS was added instead of drug solution. For the 1-hr exposure to drugs, the cells were washed two times after 1-hr incubation and resuspended in 1 ml of fresh medium to incubate for 4 days in 37°C humidified atmosphere of 5% CO_2 in air. For continuous exposure, the cells were incubated with drugs during the entire assay period. Acetaldehyde-prefixed DRBC (5×10^3) (2) were added to each tube and cells were stained for 10 mins with 1:1 mixture of 1% Fast green (Matheson Co, East Rutherford, NJ) and 1% Nigrosin (Matheson) cytocentrifuged on slides, and counterstained with Harris' formula hematoxylin-eosin (H-E; Harleco, Gibbstown, NH) or Wright-Giemsa (Harleco). The slides were then dehydrated and mounted. Living tumor cells, which exclude Fast green and Nigrosin, were stained pink with H-E or light blue with Wright-Giemsa and dead cells were stained dark green or black. The DRBC were easily identifiable by the typical morphology of small nucleated microelliptocytes. The surviving fraction was calculated by dividing the ratio of living cells over DRBC with the ratio of the control (without drug) over DRBC. All assays were performed in duplicate and results were presented as means.

FCM Assay

To dispersed tumor cells, DRBC (5×10^4) were added and mixed well. Cells were then centrifuged and resuspended in 0.5 ml of PBS and kept on ice. Propidium iodide (PI; Sigma) solution (5%) was added immediately prior to FCM analysis. A Coulter Epics V cell sorter, model 741, was used for analysis with the following filter set-up: a neutral density filter, 1.5 Å, at the narrow angle light sensor; a 488 diachronic mirror to split 90° light scatter to its detector; a 510-nm interference filter to remove residual laser light from the scattered light; and a 560-nm diachronic mirror to split light to the red photomultiplier tube. A 488-nm line at 300 mW generated by a coherent argon laser was used to illuminate the cells. As an initial step, control tumor cells were run in order to determine the area where living cells were located, in a two-parameter analysis of narrow angle (cell size) versus 90° light scatter. This area was gated by bit-mapping for live cells, with gating on the integral red signal (PI fluorescence) to remove duck and dead cell signals from the live cell area. Simultaneously, a two-parameter histogram of integral red signal versus narrow angle light signal was collected and used to determine the number of DRBC in the amount of sample analyzed (the DRBC nuclei stained in a predictable and different area from the live and dead cells and cellular debris).

Statistical Analysis

Data were analyzed by SAS (Statistical Analysis System, Inc) procedures (7) at the National Institutes of

Health Computer Utility, Bethesda, MD. Linear regression analyses between resulting surviving fractions obtained by the manual method and FCM were performed on all assays done with the cell lines and fresh leukemic cells. Analysis of covariance and multiple analysis of covariance (using the SAS General Linear Models procedure that allows for unequal subsample sizes as well as missing values) were performed to search for differences in intercepts and slopes of regression lines analyzed according to different cell sources and drugs. Both the parametric Pearson and nonparametric Spearman correlation coefficients were calculated for the fractions obtained for different drugs.

RESULTS

Cell Lines

The SCLC line NCI-H209 was well dispersed when judged by microscope after this enzymatic treatment and was tested for drug sensitivity. In the FCM method, fewer viable cells were present in control H209 cells than those of other cell lines. However, the survival fraction with different concentrations of drugs agreed well with manual counting (figs 1A and 1B). H209 was sensitive to both etoposide and doxorubicin.

The adherent NSCLC cell line H460 was trypsinized before incubation with doxorubicin and evaluated after enzymatic treatment, showing resistance with both manual and FCM counting (fig 1B). The contours of control living tumor cells were demonstrated clearly by light scattering in narrow angle and in 90° angle (fig 2A). With narrow angle light scattering shown on the x axis and red fluorescence on the y axis, the DRBC and viable tumor cells could be demonstrated in clearly separated areas (fig 2B).

HL60, growing as a single-cell floating suspension, was examined without enzymatic treatment, and aggregated cells were not observed on slide preparations. The surviving fractions obtained for the cell lines by FCM and manual methods were plotted against logarithmic drug concentrations (fig 1). Further, the regression analysis between surviving fractions obtained by FCM and manual methods is shown in figure 3A.

Leukemic Cells From Patients

Leukemic cells isolated from PBC and BMC were examined separately against various drugs mentioned in the Materials and Methods section. Surviving cell fractions were assessed both manually and with FCM. The cell sorter counted approximately 10^5 cells/min, whereas approximately 200 cells (living tumor cells and DRBC) were counted manually on each slide. In this report, we have focused on the correlation between the results obtained with those two methods. Therefore, results were pooled and regression analyses were performed (fig 3B).

Regression Analyses

Results for the cell lines are shown in figure 3A (linear regression equation: manual method = 0.00 + 0.99 FCM; r = 0.958, n = 17, $P < 0.0001$). Results for fresh leukemic cells are shown in figure 3B (manual method = −0.02 + 0.75 FCM; r = 0.831, n = 116, $P < 0.0001$). Manual counting generally showed lower values (mean: 0.355 ± 0.341, n = 137) than the cell sorter (mean: 0.503

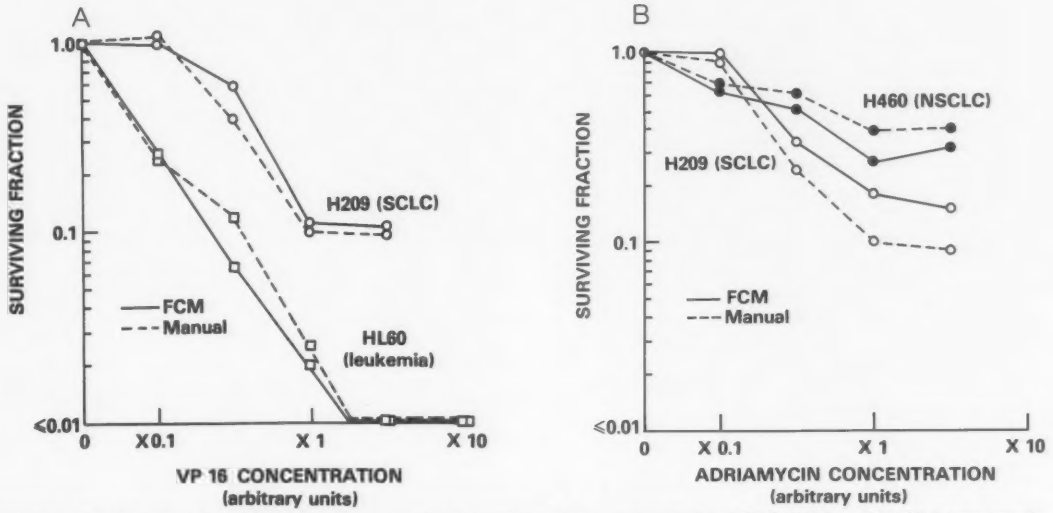

FIGURE 1.—Surviving fractions by different drug concentrations evaluated by manual method and FCM. (A) Etoposide (VP 16) was tested on a promyelocytic leukemia cell line HL60 and a SCLC cell line H209, and (B) doxorubicin (Adriamycin) was used on the NSCLC cell line H460 and SCLC line H209.

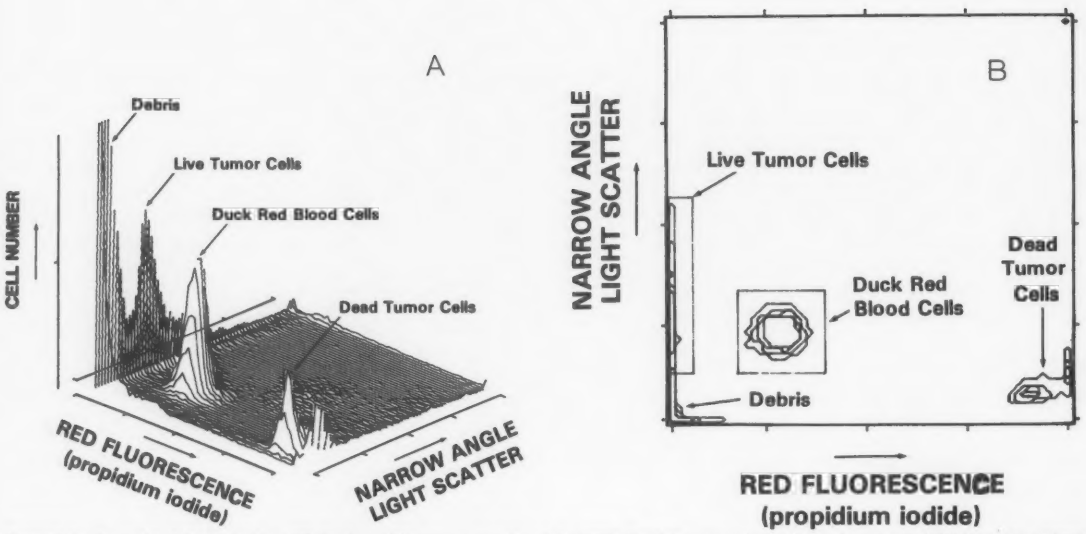

FIGURE 2.—Isometric (A) and contour (B) displays of the same experiment by FCM. Peak 1 represents the living tumor cells (SCLC cell line H209), Peak 2 represents duck red blood cells, and Peak 3 represents part of the dead tumor cell mass in G1 phase. Most of the dead cells on x axis are off the scale and are not demonstrated in the illustration.

$\pm$ 0.377, n = 117). The FCM measure was designated as the independent variable, "predicting" the manual measure as the dependent variable. Analyses of covariance displayed significant differences in slopes between BMC and PBC ($P < 0.01$) but not in intercepts. Further, significant differences between slopes were found between the two time conditions (1-hr exposure and continuous exposure: $P < 0.0002$), but, again, not in intercepts. In a multiple analysis of covariance performed to analyze which slope difference was the most important (BMC-PBC, 1-hr exposure-continuous exposure, the three patients, and the 11 different drug treatments), the result clearly showed the BMC-PBC difference to be the most significant ($P < 0.001$); drug treatments implied no significant difference at all. Knowledge of FCM cell survival rate accounted for 69.1% of the variance in the manual survival rate (*see* above). Adding knowledge of the BMC-PBC class (in an analysis-of-covariance model) increased the percentage accounted for to 78.5%, a significant increment. Further, adding all four conditions above increased the percentage of variation accounted for to as much as 90.5%.

DISCUSSION

In this paper, we have shown the feasibility of automatizing the dye exclusion assay. Thus, the comparison between manual microscopic and automated cell sorter evaluation correlates well with both fresh tumor samples and cell lines. Automatization of the analysis would increase the capacity to test more patients and more drugs, allowing rapid accumulation of data from prospective studies and correlation of clinical responses of patients to drugs selected by in vitro testing. In our department, tumor tissues from SCLC and NSCLC protocol patients are examined for in vitro sensitivity against a series of drugs (8).

With cell lines growing as floating aggregates, the cell sorter generally counted fewer viable cells than were counted manually, due to the omission of all aggregated cells by the machine. On slides, an aggregate of a few living cells could be counted easily. Despite this, the ratios of surviving fractions agreed well, probably because the controls had an equal proportion of aggregates. Furthermore, since the enzymatic dispersion of aggregated cells was carried out just before harvesting, cell-cell interactions were preserved, which may have encouraged normal growth during incubation.

For fresh BMC samples from leukemic patients, the FCM counted more living cells than the manual method. One relevant difference might be that > 90% of the BMC are blast cells, as compared with about 80% for the PBC. The blast cells in BMC and PBC may differ in quality of cell membranes, which upon slight injury may permit trypan blue dye to penetrate while being more resistant against PI dye. Correlation with clinical response of the patients may indicate the reliability of these methods.

Lung cancers and other solid tumors contain varying numbers of stromal cells. Careful discrimination of the tumor cells might then be necessary. In this aspect, the manual method may be preferred because of the possibility of the visual distinction between those cells. However, in some tumor samples (such as certain acute leukemias) which consist almost entirely of malignant cells, the direct FCM counting method described herein

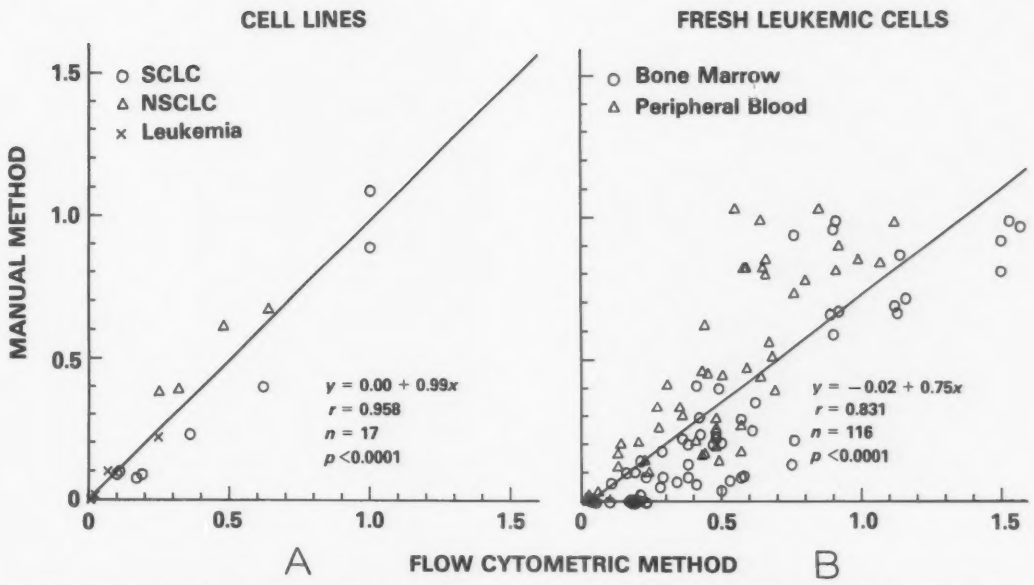

FIGURE 3.—Regression analysis showing prediction of manually counted cell survival (y axis) from the FCM-counted values (x axis) for cell lines (A) and fresh leukemic cells (B). Symbols include the results for all different drugs, patients, and duration of exposure to drugs. The correlation coefficient was significant in all divisions of the sample by cell source, duration of exposure, individual patients, and different drugs.

could be employed. In the lung cancer clinical trials currently under way at the National Cancer Institute, we usually amplify the tumor cells in vitro by short-term culture in selective media prior to testing by the manual method (8–10). This results in growth of healthy, viable tumor cells without significant stromal cell contamination. The FCM method could be utilized for testing these short-term cultures. In other cases, normal and malignant cells may have to be distinguished by the use of light scatter profiles, DNA content, or surface antigen expression. Thus, antibodies which bind either tumor cells or nontumor cells, coupled with green fluorescence such as fluorescein isothiocyanate, can be used to gate out the nontumor population. In that way, tumor cells can be differentially counted.

We have demonstrated that the automatized FCM method is compatible to the laborious and subjective manual dye exclusion assay. The FCM method may be used for individual drug therapy selection using certain tumor samples and short-term cultures. It may also be used for preclinical therapy testing utilizing continuous cell lines. We believe that further development of automatized dye exclusion assay would promise a simple, rapid, inexpensive drug-screening method with a high success rate.

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Aminoglutethimide Without Hydrocortisone in the Treatment of Postmenopausal Patients With Advanced Breast Cancer[1]

Klaus Höffken,[2,*] Herbert Kempf,[2] Antonius A. Miller,[2] Brigitte Miller,[2] Carl G. Schmidt,[2] Peter Faber,[3] and Hans K. Kley[3,4]

In a phase II study, 38 evaluable patients with advanced progressive breast cancer were treated with 4 × 250 mg of aminoglutethimide per day without hydrocortisone supplementation. Partial remissions were observed in five patients (13%), with a median duration of 9 months (range, 7–15), and stable disease was observed in 17 patients (45%), with a median duration of 4 months (range, 2–11). The recorded side effects (CNS, cutaneous, gastrointestinal) were tolerable and transient and required drug discontinuation in only four patients. Serial determinations of serum hormone levels during aminoglutethimide treatment revealed a significant decrease in estradiol and an increase in gonadotropins, testosterone, and progesterone. No patients had adrenal insufficiency. In contrast, despite prolonged aminoglutethimide treatment and persisting hypoestrogenemia, both cortisol levels and adrenal responses to ACTH remained unaltered. It is concluded that aminoglutethimide alone is effective in the treatment of postmenopausal patients with breast cancer. Moreover, based on the present findings, a prospective randomized study is warranted to show that hydrocortisone supplementation appears to not be mandatory, either for increase in efficacy or for decrease in side effects of the treatment. [Cancer Treat Rep 70:1153–1157, 1986]

Aminoglutethimide was originally introduced as an anticonvulsant drug, but was withdrawn after it was reported to induce adrenal insufficiency (1). Subsequent investigations suggested that aminoglutethimide inhibits several steps of adrenal steroid synthesis (2) and the aromatase enzyme-mediated peripheral conversion of adrenal androgens into estrone (3). This indicated that aminoglutethimide may be useful as an antiestrogen in the treatment of breast cancer, and a number of clinical trials have shown that aminoglutethimide is effective in the treatment of advanced postmenopausal breast cancer. In these trials, objective response rates of 30%–50% were achieved, which are similar to those obtained by surgical adrenalectomy or tamoxifen (4–8). However, in most studies aminoglutethimide has been used in combination with hydrocortisone in order to avoid hypocorticism and also to prevent the development of a hormonal "escape phenomenon" induced by a reflex rise in ACTH secretion (9). Such a rise would lead to a secondary increase of estrogens, thus diminishing therapeutic efficacy of aminoglutethimide.

Since more recent investigations have demonstrated that aminoglutethimide preferentially blocks androgen aromatization and leaves other adrenal enzyme systems less affected (10), it was of interest to investigate the therapeutic value and endocrine effects of aminoglutethimide administered without hydrocortisone to patients with advanced postmenopausal breast cancer.

PATIENTS AND METHODS

Patients

Forty-seven patients with advanced breast cancer entered a phase II study with aminoglutethimide. Drug dosage was gradually increased as follows: 250 mg twice daily on Days 1 and 2; 250 mg three times daily on Day 3; and 250 mg four times daily thereafter. All patients were either postmenopausal or surgically

[1] Received Oct 23, 1985; revised May 2, 1986; accepted June 3, 1986.

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[4] We thank Drs. R. Callies and G. Benker for their advice in interpreting hormonal data, and the Ciba-Geigy Company, Wehr, Federal Republic of Germany, for providing aminoglutethimide.

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oophorectomized and had previously received multiple hormonal and/or cytostatic treatment.

Aminoglutethimide treatment was initiated only in patients who were at least 2 weeks off any tumor-specific treatment and exhibited unequivocal evidence of progressive metastatic disease.

Except for irradiation of local bone mestastases, no concomitant tumor-specific treatment was allowed. In cases requiring irradiation, the local responses were excluded from evaluation of the overall response.

Response Criteria

Responses were assessed according to the International Union Against Cancer guidelines (11). Partial remission was defined as either a 50% decrease in the sum of products of all measurable lesions or the recalcification of lytic lesions developing in absence of new lesions. Stable disease (no change) was defined as patients with isolated bone lesions who did not show disease progression or new lesions and who also had subjective relief of bone pain for at least 2 months. Progressive disease was defined as the development of new lesions or an increase in old lesions. Response duration was defined by the interval between the first demonstration of disease regression or stabilization and the first documentation of disease progression.

Clinical Evaluation and Hormone Assays

Case histories and physical examinations (including blood pressure, complete blood counts, and serum electrolytes, as well as liver, renal, and endocrine function studies) were performed weekly for the first 6 weeks of treatment and monthly thereafter. All patients were followed until disease progression. Patients received papers stating that in case of unexplained hypotension, hydrocortisone should be given immediately. Moreover, they received a patient card listing the side effects of aminoglutethimide. This card, which was filled in weekly by the patient, contained a scoring index ranging from no side effects to symptoms of high degree. The degrees of toxicity were defined as follows: *low*—symptoms were perceivable but did not inhibit general activity; *medium*—symptoms reduced the usual amount of daily activity; and *high*—symptoms forced the patient to stay in bed more hours than before initiation of treatment.

Using commercially available radioimmunoassays, serum levels of the following hormones were determined: estradiol, progesterone, testosterone, dehydroepiandrosterone sulfate, luteinizing hormone, follicle-stimulating hormone, and cortisol. In addition, in six patients the cortisol response to 250 μg of an ACTH analogue (Synacthen, Ciba-Geigy Co, Wehr, Federal Republic of Germany) was tested prior to, and after 1 and 6 weeks of, treatment. For this, blood samples were collected from resting patients before and after 30, 60, and 90 minutes of ACTH injection.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	47
Evaluable	38 (81)
Mean age in yrs (range): 58.8 (37-76)	
Estrogen receptor status	
Positive	10 (26)
Unknown	24 (63)
Negative	4 (11)
Site of metastases	
Bone	38 (100)
Locoregional	18 (47)
Pleuropulmonary	14 (37)
Liver	9 (24)
Brain	2 (5)

Statistical Analysis

Statistical evaluation of the results was performed by using the Mann-Whitney U-Test.

RESULTS

The major clinical characteristics of the 47 patients who entered this trial are outlined in table 1. While all 47 patients were evaluable for toxicity, 38 patients (81%) met the criteria for response evaluation. In the remaining nine patients, response to aminoglutethimide was not evaluable because of (*a*) rapid disease progression during the first 6 weeks of treatment in six patients; (*b*) addition of hydrocortisone to aminoglutethimide for reasons other than adrenal insufficiency in two patients; and (*c*) aminoglutethimide dose reduction due to marked mental confusion in an 82-year-old patient after the first 3 days of treatment.

The mean age of the patients was 59 years and the mean Karnofsky performance status was 80%. In most cases, the estrogen receptor status of primary or metastatic lesions was unknown. A notable predominance of metastatic bone lesions was observed and, in addition, 50% of the patients exhibited locoregional and pleuropulmonary metastases.

All patients had been extensively pretreated with hormone and/or cytostatic therapy.

Response to Therapy

Table 2 shows the overall response, including the 95% confidence intervals, to aminoglutethimide of the 38 evaluable patients. Partial remissions lasting 7–15 months were observed in 13% of the patients and stabilizations of a previously progressive disease with a median duration of 4 months (range, 2–11) were induced in 45% of the patients. Forty percent of the patients failed to respond to aminoglutethimide.

Figure 1 illustrates the interval between initiation of treatment and documentation of disease progression for patients experiencing partial remission (median, 12.5 months) or disease stabilization (median, 6.5 months)

TABLE 2.—Response to aminoglutethimide treatment without hydrocortisone supplementation

Results	No. of patients (%) (95% confidence interval*)	Duration of response (mos)		
		Median	Mean	Range
Partial remission	5 (13) (5%–29%)	9	10.6	7–15
Stable disease	17 (45) (26%–68%)	4	5.3	2–11
Progressive disease	16 (42) (26%–66%)	—	—	—

*Poisson distribution.

from aminoglutethimide. By comparing median response duration and median time to treatment failure, it can be deduced that responses were encountered after a median treatment time of 2.5–3.5 months.

In table 3, responses to aminoglutethimide treatment are related to estrogen receptor status and to sites of metastases. It can be seen that patients with unknown receptor status did not respond less than those having positive estrogen receptors (> 10 fmols/mg of cytosol protein). In addition, two patients with a negative receptor status responded to aminoglutethimide. In confirmation of previous reports (5,7), bone lesions responded well in terms of both partial remission and disease stabilization. In contrast, however, locoregional metastases showed the lowest responses in the present trial.

Side Effects

Table 4 shows that only 13% of all patients had no therapy-related side effects. The majority developed weariness of medium or high degrees. One-third of the patients displayed cerebellar and gastrointestinal symptoms, and transient skin rashes of various degrees were noticed in 23%. However, despite continuation of therapy, most of these side effects subsided spontaneously after 6 weeks. Aminoglutethimide treatment had to be discontinued in only four patients due to intolerable lethargy or skin rashes. Neither hypotension nor orthostatic symptoms were encountered. In spite of the observed increase in serum testosterone (*see* below) no hirsutism was seen.

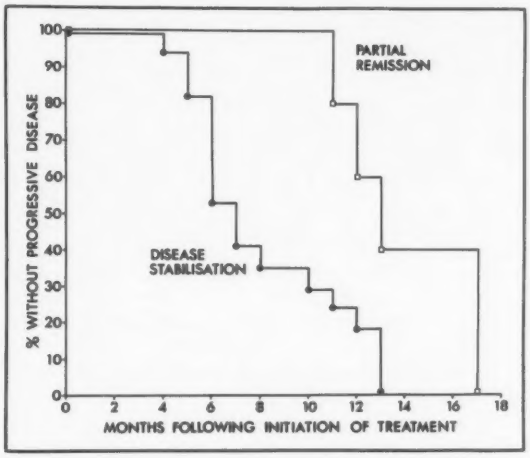

FIGURE 1.—Time to treatment failure of patients with partial remission or disease stabilization receiving aminoglutethimide without hydrocortisone supplementation.

TABLE 3.—Response to aminoglutethimide treatment without hydrocortisone supplementation in relation to receptor status and sites of metastases

	No. of patients (%)			
	Total	Partial remission	Stable disease	Progressive disease
Estrogen receptor status				
Positive	10	1	3	6
Unknown	24	3 (13)	13 (54)	8
Negative	4	1	1	2
Site of metastases				
Bone	38	5 (13)	17 (45)	16
Locoregional	18	1 (6)	4 (22)	13
Pleuropulmonary	14	4 (29)	6 (43)	4
Liver	9	1 (11)	2 (22)	6
Brain	2	—	—	2

Hormone Studies

The serum hormone levels recorded in patients receiving aminoglutethimide are shown in table 5. While cortisol and dehydroepiandrosterone sulfate remained unchanged, estradiol decreased after 6 weeks of treatment. The latter was accompanied by an increase in gonadotropins, progesterone, and testosterone, respectively. These hormonal changes persisted for at least 15 months, which was the longest treatment time in the present study (data not shown). In addition, as outlined in table 6, a normal cortisol response to iv ACTH injection was found after both 7 and 42 days of treatment.

Other Blood Examinations

Besides the known increase in gamma-glutamyl-transpeptidase, no alterations in blood chemistry were observed, either in whole blood cell counts or in serum electrolytes (data not shown).

TABLE 4.—Side effects of aminoglutethimide treatment without hydrocortisone supplementation*

	Total	Degree of toxicity†		
		Low	Medium	High
Weariness	62	31	41	28
Ataxia	32	60	13	27
Nausea	26	58	25	17
Rash	23	55	27	18
No side effects	13			

*Values = % of patients.
†For definition, *see* the Patients and Methods section.

TABLE 5.—Mean serum hormone levels ± SEM before and during aminoglutethimide treatment without hydrocortisone supplementation*

Hormone	Week 0	Week 1	Week 6	*P* values
LH (mIU/ml)	70.1 ± 14.1	88.3 ± 17.0	100.1 ± 11.9	0.02
FSH (mIU/ml)	90.5 ± 21.8	96.8 ± 25.0	151.2 ± 18.7	0.01
DHEAS (μg/ml)	0.85 ± 0.17	0.7 ± 0.09	0.67 ± 0.09	NS
Progesterone (ng/ml)	0.35 ± 0.04	0.56 ± 0.1	0.5 ± 0.07	0.10
Testosterone (ng/ml)	66.9 ± 13.3	107.1 ± 15.2	148.0 ± 25.0	0.01
Estradiol (pg/ml)	10.9 ± 0.9	10.9 ± 2.2	6.9 ± 0.6	0.0001
Cortisol (μg/dl)	18.6 ± 1.4	19.0 ± 1.4	16.3 ± 1.3	NS

*LH = luteinizing hormone, FSH = follicle-stimulating hormone, DHEAS = dehydroepiandrosterone sulfate, and NS = not significant.

DISCUSSION

The present study has shown that aminoglutethimide alone is effective in treating postmenopausal patients with advanced breast cancer. The 13% objective remission rate observed here appears to contrast with the average 31% remission rate reported by others using aminoglutethimide plus hydrocortisone (4–8). However, it should be noted that the confidence limits of response rates in most phase II studies are large. Thus, a 95% confidence interval of 5%–29% objective remissions found in the present trial does not necessarily suggest that aminoglutethimide alone is less effective than aminoglutethimide plus hydrocortisone.

Furthermore, the apparent discrepancy in objective remissions may be explained by the pretreatment characteristics and by the high incidence of bone metastases among the patients who entered the present trial. The latter notoriously decreases partial remission rates when conventional criteria of recalcification are used. Therefore, Coombes et al (12) suggested the inclusion of treatment-induced stabilization of bone metastases into the group of responders. By this, a 58% response rate is obtained in the present study, which compares favorably with previously reported response rates achieved with aminoglutethimide plus hydrocortisone (4–8).

In addition, it was found that the rate and degree of side effects caused by aminoglutethimide alone appeared not different from those observed after aminoglutethimide plus hydrocortisone. Accordingly, the frequency of severe side effects requiring drug discontinuation in the present study was similar to that of other trials employing hydrocortisone supplementation (5,7,8). However, there were still 87% of patients exhibiting tolerable side effects during the first 6 weeks of treatment. Although this number differs from the 40% side effect rate obtained in a trial involving hydrocortisone (7), it is similar to that reported by Santen et al (5). In their study, 48% of the patients developed lethargy and 33% developed skin rashes despite using hydrocortisone.

Moreover, using the same scoring system, we observed a 70% rate of no side effects in 50 patients treated in a recent phase II study with 250 mg of aminoglutethimide twice a day without hydrocortisone supplementation (manuscript in preparation).

Thus, there appears to be no conclusive evidence as yet that hydrocortisone ameliorates aminoglutethimide-related side effects. Nevertheless, the results from the present study have to be regarded as preliminary, awaiting proof by a randomized phase III study.

The interference of aminoglutethimide with adrenal steroid biosynthesis is outlined in figure 2. According to the findings of others (3,9,10,13,14) and ourselves, neither 20.22 desmolase nor 3βol dehydrogenase or 21 hydroxylase is significantly inhibited as evidenced by a secondary rise of the respective intermediate products. However, despite an increase in respective precursors, 11β hydroxylase end products remain constant, indicating that this enzyme is inhibited (but notably not blocked). By contrast, the aromatase enzyme system is blocked as indicated by the persistent suppression of estrogen levels despite increased serum testosterone. This mechanism of action is further supported by the results of the ACTH provocation test. Thus, while cortisol (table 6), 17-OH progesterone (10), and androstenedione (10) show the expected increase after ACTH injection, estrogen levels remain suppressed (10). In this context, it should be noted that in the present study aminoglutethimide alone suppressed estradiol levels by 37%, which compares well with a 30% reduction observed during treatment with aminoglutethimide plus hydrocortisone (5).

In summary, these observations indicate that aminoglutethimide acts as an antiestrogenic substance by blocking the aromatization of androgens to estrogens. The inhibition of other enzyme systems appears less important and may be overcome by reflex increases in

TABLE 6.—Response of serum cortisol to ACTH in 6 patients before and during aminoglutethimide treatment without hydrocortisone supplementation

Treatment Day	Basal value (μg/dl ± SEM)	% increase over basal value (following injection of ACTH) 30 mins	60 mins	90 mins
0	19.9 ± 1.9*	65.3	88.9	91.5
7	16.6 ± 2.1*	60.8	105.4	95.8
42	13.0 ± 1.7*	102.3	123.0	136.2

*No significant difference.

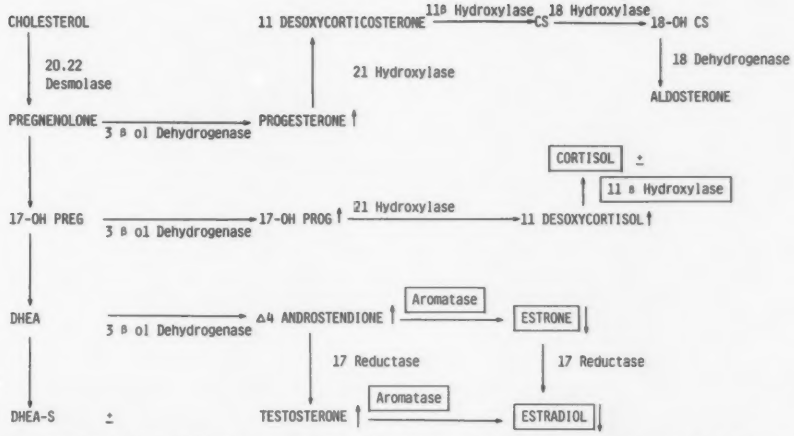

FIGURE 2.—Steroid biosynthesis during aminoglutethimide treatment (summary of data *from* refs 3, 9, 10, 13, and 14 and own findings). ↑ = increase; ↓ = decrease; and ± = no change (for further explanations, *see* text).

relevant precursors. It should be noted, however, that individual differences in acetylation of aminoglutethimide may result in drug concentrations that could also block, rather than inhibit, 11β hydroxylase. This would explain the adrenal insufficiencies developing occasionally in both children (1) and in patients with breast cancer even after low-dose treatment (15). Therefore, omission of hydrocortisone supplementation should be paralleled by regular determination of serum cortisol levels.

In conclusion, it is suggested from the present study that aminoglutethimide alone is a potent antiestrogenic drug. Hydrocortisone supplementation appears necessary only upon demonstration of a decrease in serum cortisol. Based upon this evidence, a randomized study is warranted to show that hydrocortisone neither adds to the efficacy of aminoglutethimide in the treatment of postmenopausal breast cancer nor decreases the rate or degree of side effects.

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Pharmacokinetic Interactions of Cyclophosphamide and 5-Fluorouracil With Methotrexate in an Animal Model[1]

Ernst A. de Bruijn, Oscar Driessen,* Paul Leeflang, Nel van den Bosch, Elisabeth van Strijen, Peter H. T. J. Slee, and Jo Hermans[2]

In tumor-bearing WAG/Rij rats the interaction of cyclophosphamide and/or 5-fluorouracil (5-FU) with methotrexate as manifested at the pharmacokinetic level was studied. Four groups were formed of at least ten animals. The control group, which received single-agent methotrexate, was compared with groups that received methotrexate plus cyclophosphamide, methotrexate plus 5-FU, and methotrexate plus cyclophosphamide plus 5-FU. There appeared to be an increase of 40% in the clearance of methotrexate by the triple combination. Cyclophosphamide especially diminished the terminal part of the concentration-time curve of methotrexate. [Cancer Treat Rep 70:1159-1165, 1986]

Methotrexate is a widely used agent for the treatment of several human neoplastic diseases; it is also an immunosuppressive agent (1,2).

Methotrexate is often combined with cyclophosphamide and 5-fluorouracil (5-FU) (CMF). Until now, the CMF regimen has been applied with variable success (3). Even as adjuvant therapy the CMF regimen showed no clear impact on rates for complete response and duration of survival (4). The reported steep dose-response relationship of the CMF regimen (4,5) indicates that pharmacokinetic studies on the three drugs in this regimen could give important information when related to clinical responses.

Although pharmacokinetic studies on the three drugs used as single agents have been reported, the pharmacokinetics of CMF in the treatment of breast cancer have been described only once (6); the patients in this study, who were on a fixed-dose regimen, showed large differences in the pharmacokinetics of CMF. Any mutual interactions of the drugs on their pharmacokinetics could not be detected due to a lack of comparison with single-agent therapy in the same subjects. The highest determined area under the plasma concentration-time curve (AUC) of methotrexate appeared to be fourfold the lowest AUC. For cyclophosphamide and 5-FU even larger differences between the highest and lowest AUCs were found.

From the study by Slee et al (6), the question arose whether the observed large variability in pharmacokinetics was perhaps related to an interaction of the three drugs. Another question is whether observed pharmacokinetic variability is reflected in pharmacodynamic results. An answer to these questions cannot be obtained very well from a patient study. Hence, the CMF treatment was investigated in a homogeneous strain of inbred tumor-bearing rats, allowing comparison of single-agent therapy with various combinations of cyclophosphamide, methotrexate, and 5-FU. In reporting this study here we restrict ourselves to the problem of interactions at the pharmacokinetic level and moreover to methotrexate, either used as a single agent or in combinations.

MATERIALS AND METHODS

Drugs

Methotrexate was supplied by Pharmachemie B. V. (Haarlem, The Netherlands); cyclophosphamide was supplied by ASTA (Bielefeld, Federal Republic of Germany); and 5-FU was supplied by Hoffmann-La Roche (Mijdrecht, The Netherlands).

Animals and Drug Administration

Female WAG/Rij rats with two implanted tumors of about 1 cm^3 in both flanks and weighing 190 ± 10 g were cannulated under light ether anesthesia 1 day before treatment with methotrexate or combinations with cyclophosphamide and 5-FU. From cannulation until the end of the kinetic experiments the rats were fasted, while water was supplied ad libitum. The flex-

[1] Received Nov 9, 1984; revised May 12, 1986; accepted June 9, 1986.

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ible cannulas, filled with a saline solution containing a plug of heparin (200 IU) at the end of the cannula, were inserted into the carotid arteries.

During the experiments the rats were kept in cages in which free movements were possible despite the cannulation. Each cage contained one rat; the cages were placed in a temperature-controlled room at 20°C and illuminated from 7:00 AM until 1:00 PM. Drug treatment was started at 9 AM. Methotrexate and combinations of methotrexate with cyclophosphamide and 5-FU were administered to at least ten rats per treatment group. Treatment modalities are indicated by M (single-agent methotrexate), MC (methotrexate + cyclophosphamide), MF (methotrexate + 5-FU), and MCF (methotrexate + cyclophosphamide + 5-FU).

Methotrexate (2 mg/kg) and 5-FU (20 mg/kg) were administered by intra-arterial injection; immediately after administration the cannula was rinsed with 200 μl of saline solution. Cyclophosphamide (70 mg/kg), dissolved in 1 ml of water plus 50 μl of ethanol, was administered orally by stomach tube. The doses were chosen in such a way that more or less comparable plasma concentration-time courses were achieved in rats as observed in patients. The sequence of administration was always (*a*) cyclophosphamide, (*b*) methotrexate, and (*c*) 5-FU. The maximal number of rats that could be treated in 1 week was eight. Treatment modalities were equally spread over the whole period of the experiment.

Collection of Blood Samples

Via the cannula in the carotid artery, 600 μl of blood was collected in polythene tubes at 0.08, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, and 8.0 hours after drug administration, which resulted in a total blood loss of 5.4 ml. To compensate for this, blood transfusions (1 ml) were given after each second sample. The blood for transfusion was taken fresh from a female WAG/Rij rat. Blood samples were centrifuged at 1000 × *g* for 10 minutes and plasma was stored at −35°C until analysis.

Assay of Methotrexate

Methotrexate plasma concentrations were determined by an enzymatic assay described by Overdijk et al (7), which is based on the capacity of methotrexate to inhibit the enzyme dihydrofolic acid reductase. Using this enzymatic assay, cyclophosphamide, 5-FU, and/or their metabolites did not interfere with methotrexate determination in plasma. The limit of determination for methotrexate in rat plasma using a minimal amount of 50 μl was 10 ng/ml, with a coefficient of variation of 7.8%.

Pharmacokinetic Analysis

Pharmacokinetic calculations were carried out by both compartmental (NONLIN) and noncompartmental analysis, or by linear regression analysis of the terminal parts of the plasma concentration-time curves. Compartmental analysis of the data was carried out by fitting Equation 1 to derived plasma concentration-time curves and by performing weighted nonlinear regression analysis as described by Metzler et al (8):

$$C = C_1 \cdot e^{-\lambda 1 t} + C_2 \cdot e^{-\lambda 2 t} \quad [1]$$

In Equation 1, C_1 and C_2 are hybrid intercept terms and λ_1 and λ_2 are hybrid coefficients representing rate constants of the drug distribution phase and elimination phase, respectively. The reciprocal of the observed plasma value served as weighting factor for the calculations. The quality of the fittings was judged by both the scatter of actual data points around the fitting function and comparison of weighted residual errors. Distribution half-life ($t_{½,\lambda_1}$), elimination half-life ($t_{½,\lambda_2}$), AUC, and clearance (CL) were calculated from C_1, λ_1, C_2, λ_2, and dose D by:

$$t_{½,\lambda_1} = 0.69/\lambda_1 \quad [2]$$

$$t_{½,\lambda_2} = 0.69/\lambda_2 \quad [3]$$

$$AUC = C_1/\lambda_1 + C_2/\lambda_2 \quad [4]$$

$$CL = D/AUC \quad [5]$$

Elimination half-lives were also calculated by linear regression analysis of the terminal part of the log plasma concentration versus time curves; for all curves ($n = 45$), the concentration at 60 minutes after drug administration was taken as the start of the terminal part. This second estimation of elimination half-life is denoted as $t_{½,z}$. The mean residence time (MRT) was calculated according to the method of Riegelman and Collier by the following equation:

$$MRT = \frac{\int_0^\infty t \cdot Cp\ dt}{\int_0^\infty Cp\ dt} = \frac{AUMC_\infty}{AUC_\infty}, \quad [6]$$

in which AUMC represents the area under the moment curve with extrapolation to infinity (9).

The volume of distribution (V_{ss}) was derived from:

$$V_{ss} = D \cdot AUMC/(AUC)^2 - D \cdot T \cdot /2 \cdot AUC, \quad [7]$$

in which D is the dose and T represents the time of administration. Since T was < 1 minute the second term was neglected in the calculations (10).

Statistical Tests

First, all parameters over the four different groups were compared. This was done using a one-way analysis of variance with Scheffe's procedure for multiple comparisons. Second, special attention was desired in comparing M plus MC with MF plus MCF and M plus MF

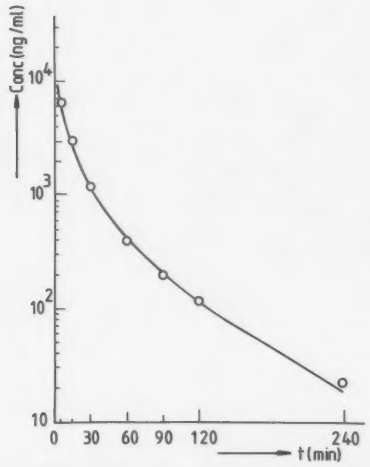

FIGURE 1.—Representative plasma concentration-time curve of methotrexate in a WAG/Rij rat following single intra-arterial injection of 400 μg of methotrexate.

with MC plus MCF; for these analyses Student's two-sample t test was used. For all tests $P < 0.05$ was used for significance level.

RESULTS

In figure 1, a representative example of a plasma concentration-time curve of methotrexate is shown for an M-treated rat.

Immediately after drug administration plasma concentrations decreased rapidly during the first 30 minutes, predominantly representing drug distribution from plasma into tissues. This phase was followed by a slower concentration decline, from approximately 500–20 ng/ml between 60 and 240 minutes, mainly symbolizing drug elimination. In plasma obtained 360 and 480 minutes after injection, generally no methotrexate could be detected. In figures 2a–2c the ranges between the highest and the lowest plasma concentrations are presented for MC, MF, and MCF, together with those for M.

The differences in figures 2a–2c clearly illustrate the difference in overlap of areas of methotrexate plasma concentration-time curves between the M-treated rats and each of the other three treatment modalities. Without exception, all plasma concentration-time curves of the M-, MC-, MF-, and MCF-treated rats could be fitted adequately by Equation 1. The most important pharmacokinetic parameters, derived by curve fittings and linear regression analysis, are shown in figure 3 for all treated rats. This figure shows some individual difference notwithstanding the rigorous standardization of the animal model.

Table 1 shows the mean pharmacokinetic data together with their standard deviations and median values. In general, relatively small differences were found between mean and median, while a tendency for a decrease in standard deviation from M- to MCF-treated rats was noted.

The significant differences between the pharmacokinetic parameters of M-, MC-, MF-, and MCF-treated rats are shown in table 2. With respect to the influence of 5-FU, a significant decrease of C_l was found (M + MC vs MF + MCF) only when the groups were combined.

Significant differences in the AUC and CL of methotrexate were found in all comparisons in which MCF-treated rats were included, except for MC versus MCF

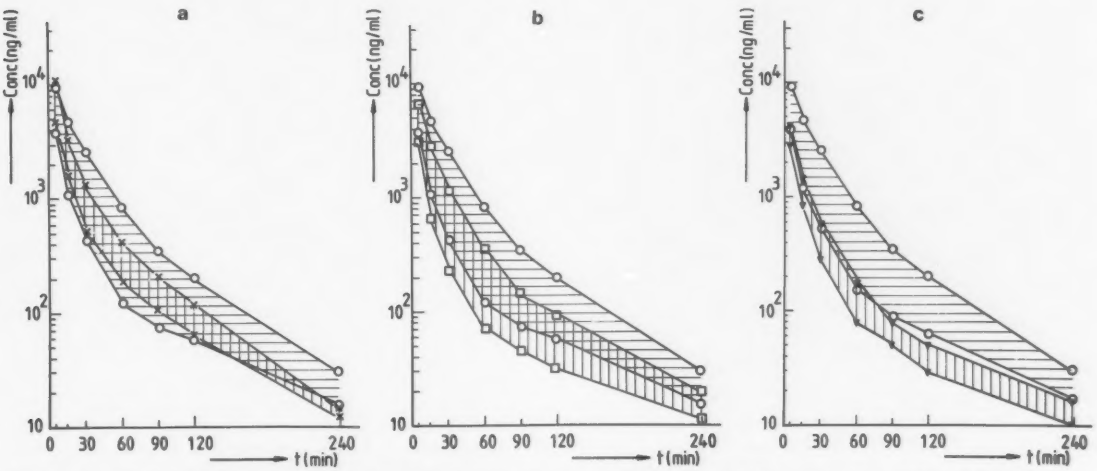

FIGURE 2.—a. Highest and lowest methotrexate plasma concentration-time curves of M-treated rats (○) in comparison to MC-treated rats (×). b. As in fig 2a: for M-treated rats (○) in comparison to MF-treated rats (□). c. As in fig 2a: for M-treated rats (○) in comparison to MCF-treated rats (▽).

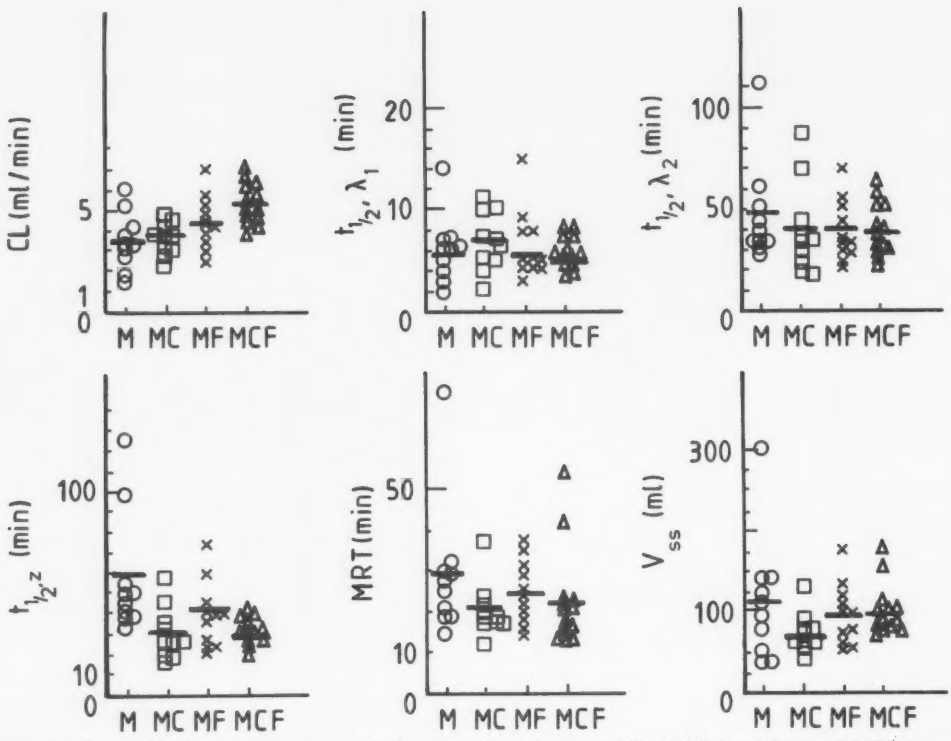

FIGURE 3.—Pharmacokinetic parameters together with their means for methotrexate of M- (○), MC- (□), MF- (×), and MCF- (▲) treated rats.

and MF versus MCF. The $t_{½,z}$ of methotrexate was significantly lower for the MC- and MCF-treated rats compared with M-treated rats and when the $t_{½,z}$ of MC plus MCF-treated rats was compared with the $t_{½,z}$ of M plus MF-treated rats.

DISCUSSION

Pharmacokinetic Interactions

From figure 2 and table 2 it can be inferred that the combination of cyclophosphamide and 5-FU interacts with methotrexate, giving rise to different pharmacokinetics. This can be caused by changes of distribution, elimination, and/or metabolism of methotrexate. Using the enzymatic assay to measure methotrexate concentrations, an influence of the cross-reactivity (1%–10%) of metabolites of methotrexate in the assay, eg, 7-OH methotrexate, cannot be excluded. However, in rats only relatively small amounts of 7-OH methotrexate are found (11).

Hence, an alteration in the amount of metabolites will translate itself only for a minor part in the results of the enzymatic assay. By measuring methotrexate and some of its metabolites as the activity on the target enzyme, relevant estimation of changes of methotrexate *activity*, caused by cyclophosphamide and 5-FU, is obtained. In the following we will interpret the results of the measurements of the enzymatic assay as plasma concentrations of methotrexate.

The effect of cyclophosphamide on the pharmacokinetics of methotrexate is mainly expressed in the final part of the methotrexate plasma concentration-time course (parameter $t_{½,z}$), whereas the influence of 5-FU manifests itself in the first part of the methotrexate plasma concentration-time curve (parameter C_1). Both drugs accelerate the disappearance of methotrexate from plasma.

Cyclophosphamide

Interactions of cyclophosphamide with methotrexate have been described for hepatic uptake and renal excretion of methotrexate (12). Recirculation of methotrexate due to liver uptake and biliary excretion was reported; cyclophosphamide was demonstrated to be capable of influencing this process. Steinberg et al (13) described a balance between renal excretion and hepatic uptake and biliary excretion. In rats with seriously impaired renal function and disturbed enterohepatic circulation by biliary diversion, a combined (double) loss of two re-uptake possibilities occurs: biliary excretion and subsequent gastrointestinal uptake and tubular reabsorption are

TABLE 1.—Median, mean, standard deviation, and coefficient of variation (CV) for the most important pharmacokinetic parameters of methotrexate obtained from M-, MC-, MF-, and MCF-treated rats

Parameter	M (n = 10)	MC (n = 10)	MF (n = 11)	MCF (n = 13)
C_1 (μg/ml)				
Median	9.5	9.4	7.9	7.5
Mean	11.2	9.6	8.0	7.5
SD	5.6	4.8	2.9	2.2
CV (%)	50	50	36	29
C_2 (μg/ml)				
Median	681	645	596	606
Mean	725	728	655	641
SD	297	626	415	350
CV (%)	41	86	63	55
AUC (ng/ml × min)				
Median	138	122	98	79
Mean	130	122	104	82
SD	56	33	32	16
CV (%)	43	27	31	20
CL (ml/min)				
Median	3.4	3.8	4.3	5.0
Mean	3.5	3.8	4.1	4.9
SD	1.4	0.9	1.3	1.0
CV (%)	40	24	32	20
$t_{½,\lambda 1}$ (mins)				
Median	6.0	7.0	5.0	5.0
Mean	5.9	6.8	6.4	5.3
SD	3.2	2.9	3.5	1.7
CV (%)	54	43	55	32
$t_{½,\lambda 2}$ (mins)				
Median	34	35	33	36
Mean	48	40	39	38
SD	25	22	15	12
CV (%)	52	55	38	32
V_{ss} (ml)				
Median	104	63	100	87
Mean	113	72	93	98
SD	77	25	40	31
CV (%)	68	35	43	32
$t_{½,z}$ (mins)				
Median	48	28	41	28
Mean	58	32	40	30
SD	29	13	17	6
CV (%)	50	41	43	20
MRT (mins)				
Median	27	19	24	20
Mean	29	21	24	22
SD	17	7	8	12
CV (%)	59	33	33	55

diminished. This resulted in a marked decrease of the plasma concentration (13).

Studies in the WAG/Rij rats revealed that cyclophosphamide was rapidly absorbed from the gastrointestinal tract and was subjected to hepatic metabolism within 5 minutes (14). This implies that influences on the renal function by active metabolites of cyclophosphamide might manifest themselves in a decrease of methotrexate reabsorption in the distal tubule within the first hours following coadministration with methotrexate (15). This and the influence on hepatic uptake and excretion might result in a more rapid elimination of methotrexate. Furthermore, interferences with the metabolism of methotrexate are among the possibilities, as has been described for several antibiotics (16).

5-Fluorouracil

Interactions of 5-FU with methotrexate at the cellular level have often been described (17-24). Apparently, an interaction at the pharmacokinetic level can also take place. From tables 1 and 2 it can be deduced that coadministration of 5-FU can influence the distribution phase of methotrexate. This can be seen by comparing

TABLE 2.—Significant differences between pharmacokinetic parameters of treatment groups*

	Parameter			
	C_1	AUC	CL	$t_{½,z}$
M vs MC	—	—	—	S
M vs MF	—	—	—	—
M vs MCF	—	S	S	S
MC vs MF	—	—	—	—
MC vs MCF	—	—	—	—
MF vs MCF	—	—	—	—
M + MF vs MC + MCF†	—	S	S	S
M + MC vs MF + MCF‡	S	S	S	—

*Student's two-sample t test and one-way analysis of variance with the Scheffe's procedure for multiple comparisons (S) were used. Other pharmacokinetic parameters did not show significant differences between treatment groups.

†Tests the influence of cyclophosphamide.

‡Tests the influence of 5-FU.

the C_1 of MF plus MCF-treated rats with that of M plus MC-treated rats. It seems that 5-FU exerts its effect on the distribution of methotrexate during the first hour after administration of the two drugs, probably because of the high initial concentration of 5-FU during that period. During the elimination phase of methotrexate, 5-FU concentrations were low (< 1 μM) as measured by high-performance liquid chromatography. The cause of the pharmacokinetic interaction of 5-FU with methotrexate is unclear; alterations in the uptake of methotrexate by tissues (25) and/or protein binding are among the possibilities (26,27).

Animal Model and Clinical Relevance

Animal experiments were intended to simulate the pharmacokinetics and pharmacodynamics of the drugs applied in the CMF regimen for the treatment of breast cancer patients. The plasma concentrations in the model were about the same as in patients (6). Notwithstanding the standardization of the model and the treatment, in these experiments a pharmacokinetic variability was found which was comparable with that of the patient study (6). Surprisingly, addition of cyclophosphamide and 5-FU to the treatment made the pharmacokinetic variability between individuals smaller in the animal model, as can be seen by comparing figures 2a–2c. It is not known whether cyclophosphamide and 5-FU have the same kind of effect on the variability of methotrexate pharmacokinetics in patients. In the literature a patient study can be found in which an influence of cyclophosphamide on the pharmacokinetics of methotrexate was observed. No data were presented about a diminished variability of methotrexate pharmacokinetics by co-administration of cyclophosphamide (28).

In comparing the pharmacokinetics of methotrexate in rats with those in patients, linearity of methotrexate kinetics has to be discussed. Comparing the doses of methotrexate applied in this study to the ones that induce nonlinear pharmacokinetics in the rat (29), it can be assumed that in this study the influence of cyclophosphamide and 5-FU on methotrexate occurs *at linear* behavior of methotrexate. In patients, doses of methotrexate as used in the CMF regimen also result in linear pharmacokinetics (28). In this animal model, if a correlation between the above-mentioned alterations in pharmacokinetics and alterations in pharmacodynamics can be observed, such an influence should also be considered in patient treatments.

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Enhancement of Semustine-Induced Cytotoxicity by Chlorpromazine and Caffeine in a Human Melanoma Xenograft[1]

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Chlorpromazine (CPZ) and caffeine (CFN) enhance the cytotoxicity of nitrosoureas in conventional murine tumor systems, but this effect was not confirmed in a randomized clinical trial which compared the action of semustine (MeCCNU) against the combination of MeCCNU, CPZ, and CFN. Since differences in repair systems are known to exist between cells of human or murine origin, we have employed a human melanoma xenograft system to quantify the drug interaction. The enhancement in human melanoma cells was similar to that observed with conventional murine tumor systems. Alkaline elution studies and determination of radioactivity from labeled MeCCNU pointed to increased drug retention and fixation of DNA damage as the mechanism of enhancement. Although toxicity studies were limited to murine tissues, there was evidence of increased toxicity, especially if MeCCNU was combined with both CPZ and CFN. Thus, a true therapeutic synergism may not be present for the combination. Some explanations for the failure to detect such drug interaction in clinical trials and the relevance of advanced preclinical tumor systems are discussed. [Cancer Treat Rep 70:1167-1171, 1986]

Caffeine (CFN) and chlorpromazine (CPZ) enhance the cytotoxicity of alkylating agents in various rodent transplantation tumor systems (1). These preclinical data have encouraged Cohen et al (2) to compare the action of semustine (MeCCNU) alone against the combination of MeCCNU, CFN, and CPZ in patients with disseminated malignant melanoma in a randomized clinical trial. Although a large number of patients were entered into the trial ($n = 157$), no significant differences were detected between the two treatment arms with respect to tumor response or toxicity.

Since recent investigations have uncovered major differences in content of repair enzymes (namely O^6-alkyltransferase) between normal and neoplastic tissues from humans or rodents (3), the question arises whether the enhancement is limited to rodent transplantation tumors.

Using congenitally athymic (nu/nu) mice as recipients, human melanoma tissue of defined sensitivity to nitrosoureas has been perpetuated as xenografts (4). Both treatment forms could then be compared simultaneously on these xenografted tissues.

To study the interaction between MeCCNU and the sensitizers CFN and CPZ, DNA interstrand cross-links were determined 24 hours after cytotoxic drug treatment of xenograft-bearing mice. Clinical trials and previous animal experiments have left open the question of a possible increase in toxicity by chemical sensitization. Therefore, murine bone marrow cellularity and mouse lethality were used as endpoints in order to make a judgment as to the therapeutic benefit derived from the combined treatment.

MATERIALS AND METHODS

The details of maintaining athymic (nu/nu) mice (NIH background, random-bred), the establishment of human melanoma line Str, and the response of Str to various nitrosoureas have been described previously (4). The experiments described here were performed on transplant generations 24-26. Tumor volumes (V) were calculated from sequential caliper measurements of length (L) and width (W) according to the formula:

$$V = L \times W^2 \times 0.5.$$

These values were transformed into relative values for each individual tumor-bearing animal with respect to the day of initiation of treatment, and means of each treatment group of six to nine animals were plotted. Growth delay values (GD) were calculated from tumor volume doubling times for untreated animals (T_c) and treated animals (T_t) according to the formula (4):

[1] Received Sept 23, 1985; revised May 19, 1986; accepted June 9, 1986.

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$$GD = (T_t - T_c)/T_c.$$

Toxicology studies were not performed on nude mice, since these animals suffer from deaths not due to drug exposure which are difficult to account for in the comparison of treatment groups. Rather, 4–6-week-old male NMRI random-bred mice were used for all toxicology studies.

The assessment of bone marrow cellularity also followed previously published procedures. Each treatment group consisted of 15 mice. Both femurs were excised rapidly after cervical dislocation and flushed with ice-cold physiologic saline; after dispersion, cells were carefully suspended in saline. Nucleated cells were counted with an electronic cell counter (Coulter model ZBI, Coulter Electronics Ltd, Dunstable, Beds, England).

The technique of alkaline elution, as adapted to the use of unlabeled tumor cells, has also been described previously (5). Before alkaline filter elution, necrotic cells were separated from xenografted melanomas by isopyknic density-gradient centrifugation, but they were not separated from murine bone marrow cell suspensions.

Cross-link factors (CF) were calculated from relative DNA retention on the filters loaded with untreated (r_o) and treated (r) cells after 12 hours of alkaline elution according to the formula:

$$CF = (1 - r_o/1 - r)^{1/2} - 1.$$

Retention of radioactivity from MeCCNU labeled in the chloroethyl moiety was determined 24 hours after drug administration with or without the concurrent administration of CFN and CPZ. The wet weight of tumors was determined immediately after excision and all tumors were incinerated completely in a Packard Tricarb sample oxidizer. Overall counting efficiency was determined at 70% by calibrating paper strips with 5000 dpm of ^{14}C.

Drugs

Radioactive MeCCNU, 1-(2-chloroethyl-U-^{14}C)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (specific activity, 21.5 μCi/mg), was a gift from Dr. H. Engle of the National Cancer Institute (NCI), Bethesda, MD. Nonlabeled MeCCNU was also received from the NCI and made up with 10% ethanol, 10% Emulphor, and 80% physiologic saline. The emulsion was immediately frozen at −70°C and thawed rapidly prior to injection.

Radioactive MeCCNU was diluted 15-fold with cold MeCCNU and prepared similarly. The dose of MeCCNU was 18 mg/kg in all experiments designed to measure retention of radioactivity.

CFN was obtained as a fine chemical from Merck Co, Darmstadt, Federal Republic of Germany, and CPZ was obtained as a clinical formulation for iv use (Megaphen; Bayer Co, Federal Republic of Germany).

CPZ (15 mg/kg) was always injected ip 6 hours before MeCCNU, whereas CFN (100 mg/kg) was given sc immediately before MeCCNU.

RESULTS

Figure 1 shows the enhancement of tumor shrinkage by concurrent administration of CFN and CPZ together with 3 mg/kg of MeCCNU in contrast to MeCCNU alone. A growth delay value of 11.6 was calculated for the combined treatment. Previously (4), a linear regression function was found for the growth delay induced by MeCCNU on human melanoma xenograft line Str:

$$GD = -0.47 + 1.28 \times \text{dose of MeCCNU}.$$

According to this equation, a single dose of 9.4 mg/kg of MeCCNU is required to achieve the growth delay value of 11.6 afforded by the combined treatment. From these doses, an enhancement factor of $9.4/3 > 3$ was derived. Additional experiments, however, have shown that this enhancement is somewhat smaller at lower doses of MeCCNU. Neither CFN nor CPZ alone enhanced the antitumor efficacy of MeCCNU (data not shown).

The lethality of the combined treatment was tested in four possible ways: MeCCNU alone versus MeCCNU + CFN versus MeCCNU + CPZ versus MeCCNU + CFN + CPZ. Neither CFN nor CPZ alone caused any lethality at the doses employed. A displacement of the dose response curves for MeCCNU-induced lethality was observed in the order of CFN < CPZ < CFN + CPZ (fig 2).

The enhancement factor calculated from the doses of MeCCNU alone versus MeCCNU + CFN + CPZ ranged from 2.2 at the LD90/30 level to 1.8 at the LD10/30 level. It is important to note that the degree of enhancement is a function-dependent variable on the dose of MeCCNU.

Murine bone marrow cellularity was assayed 3 days after drug treatment. The results are presented in table 1. After treatment with 18 mg/kg of MeCCNU there is

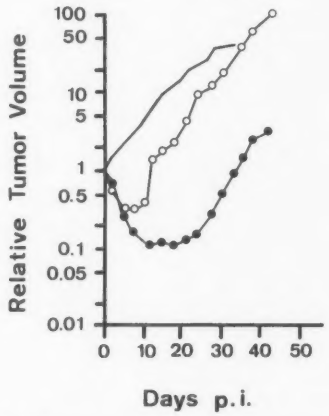

FIGURE 1.—Relative tumor volumes of melanoma xenograft line Str after treatment with 3 mg/kg of MeCCNU alone (open circles) or with CFN and CPZ (solid circles). Solid line without symbols indicates mean tumor volumes of untreated animals. p.i. = post injection.

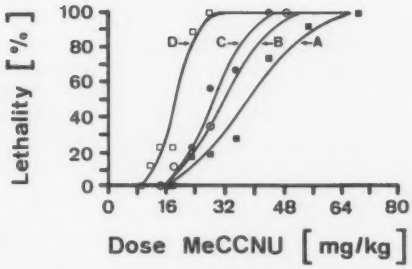

FIGURE 2.—MeCCNU-induced lethality at 30 days: A (solid squares) = MeCCNU alone; B (open circles) = MeCCNU + CFN; C (solid circles) = MeCCNU + CPZ; D (open squares) = MeCCNU + CFN + CPZ. Note the change in the slope rather than a displacement of the dose response curves. The curves were generated by probit analysis using the SAS program.

a clear reduction of bone marrow cellularity, which is enhanced by either CFN or CPZ alone but more so by the concurrent administration of CFN and CPZ.

Using the "Scheffe" test, treatment groups were compared in two ways. Considering all data on bone marrow cellularity, including untreated controls and mice receiving no MeCCNU but either CFN, CPZ, or both, a significant difference ($P < 0.001$) was detected only between mice treated with MeCCNU and those without cytotoxic treatment. By restricting this test to recipients of MeCCNU, a significant difference ($P < 0.001$) was detected only between mice receiving either one or both sensitizers and cytotoxic treatment only.

To further elucidate the enhancement of cytotoxicity from MeCCNU, the retention of radioactivity in tumors from ip injection of ^{14}C-labeled MeCCNU was assessed 24 hours after treatment in whole blood, brain, heart, liver, and xenograft tissue with or without concurrent administration of CFN and CPZ (table 2).

There was a significantly different retention in xenograft tissues, whereas this difference was not significant for whole blood, plasma, liver, heart, and brain when comparisons were made on the basis of wet weight of the tissues examined or the fluid equivalents from blood (U-test).

TABLE 1.—Nucleated cells in both femurs ($\times 10^{-6}$)*

Drug	Mean	SEM
Control	2.89	0.16
CPZ	3.28	0.16
CFN	2.90	0.13
CPZ + CFN	2.88	0.13
MeCCNU	1.07	0.10
MeCCNU + CFN	0.73	0.09
MeCCNU + CPZ	0.60	0.05
MeCCNU + CPZ + CFN	0.43	0.03

*$n = 15$.

TABLE 2.—Retention of radioactivity (dpm) per g of tissue 24 hrs after ip injection

	MeCCNU			MeCCNU + CPZ + CFN		
Tissue	Mean	SEM	n	Mean	SEM	n
Blood	2404	176	4	1911	321	8
Tumor*	1279	159	8	3226	425	16
Liver	6544	88	4	18357	8671	8
Heart	2192	79	4	2431	133	8
Brain	1049	28	4	1225	116	8

*Difference statistically significant (U-test, alpha = 0.05).

The cytotoxicity of nitrosoureas has been linked to the formation of DNA interstrand cross-links (6), which were measured in our experiments 24 hours after exposure to MeCCNU either with or without CFN and CPZ.

Figure 3 shows the alkaline elution patterns of DNA from human melanoma xenograft Str. The DNA from cells treated with MeCCNU in conjunction with CFN and CPZ apparently has sustained more cross-links than DNA from cells treated with MeCCNU alone. The cross-link factors are 0.046 and 0.098, respectively, indicating an enhancement by a factor of 2.

DNA damage was also prominent in murine bone marrow cells. In figure 4 linear regression functions are plotted for cross-link factors as a function of dose of MeCCNU with or without CFN, CPZ, or both sensitizers. The steepness of the linear regression function is increased by a factor of about 3 for the combination of

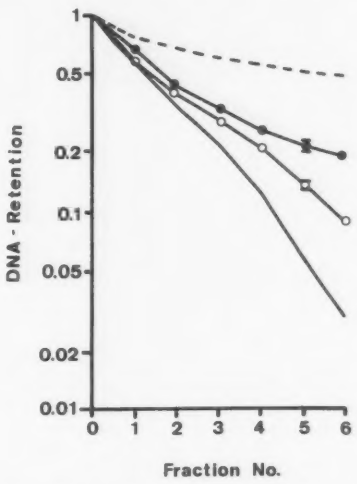

FIGURE 3.—Alkaline elution patterns from DNA isolated from xenografts 24 hrs after in vivo treatment with MeCCNU alone (open circles) or together with CFN + CPZ (solid circles). Untreated controls without 6 Gy are indicated by dashed line; untreated controls treated with 6 Gy are indicated by solid line. Experiments were performed in triplicate and bars display SEM for fraction No. 5.

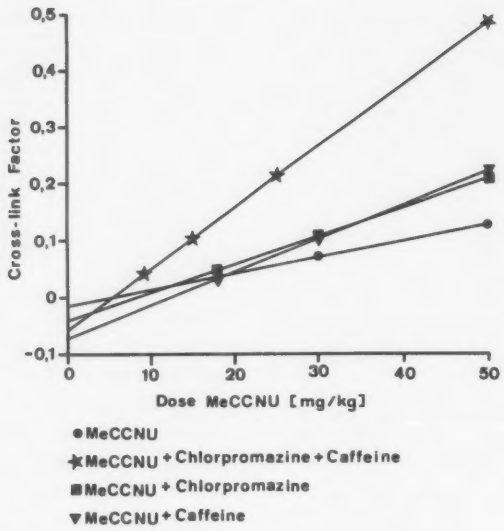

FIGURE 4.—Dose response curves obtained with murine bone marrow cells for the induction of DNA interstrand cross-links.

MeCCNU with CFN and CPZ, but remains essentially unchanged if only CFN or CPZ is combined with MeCCNU.

DISCUSSION

The data elaborated with the human melanoma xenograft line clearly demonstrate that the antineoplastic action of MeCCNU is enhanced in human tissues by concurrent administration of CFN and CPZ. Recent evidence from in vitro experiments with human bladder cells confirm that methylxanthines enhance the cytotoxicity of nitrosoureas (7).

Our data show that more radioactivity is retained in xenografted tissues after administration of ^{14}C-labeled MeCCNU, and more DNA interstrand cross-links persist after combined treatment.

Early investigations on the mechanism by which CFN enhances the cytotoxicity of alkylating agents have pointed to an inhibition of postreplicational DNA repair mechanisms (8). Recently, this hypothesis has been changed in favor of a mechanism whereby the arrest in G_2 phase of the cell cycle normally occurring after DNA damage is released in the presence of CFN (9). Transmitotic cell death then ensues from further cell cycle traverse without repair of DNA damage. The latter interpretation agrees well with the absence of a change in MeCCNU-induced DNA damage by CFN alone.

The role of CPZ is even more difficult to interpret. The drug may be cytotoxic by itself at high doses (10) and acts as a calmodulin inhibitor, which enhances the activity of anthracyclines and several other antineoplastic agents probably by interfering with intracellular drug retention (11). There are no data on selective CFN retention in neoplastic tissues, but it has been reported that CPZ is preferentially retained in melanotic tissues (12). In contrast to findings of Van Woert and Palmer (13), no single-agent activity was found for CPZ against the human melanoma Str.

According to our data, an increase both of drug retention and of macromolecular DNA damage contributes to the enhancement of MeCCNU-induced cytotoxicity by the combination of CFN and CPZ.

Our data show that macromolecular DNA damage is enhanced without specificity in normal murine and malignant human tissues; bone marrow toxicity and whole-mouse lethality are also enhanced.

DeWys and Bathina (14) describe a synergism between carmustine and theophylline, but they also report an increased lethality of normal mice. According to their work, the reduction in peripheral wbcs is greater for the combination than for carmustine alone.

Considering toxicity of the combined treatment, it should be remembered that the degree of enhancement itself is dependent on the dose of MeCCNU applied. This might explain some of the disputes about therapeutic synergism achievable with chemosensitizers.

Reviewing the evidence from experimental tumor systems, including human tumor cells propagated either as xenografts or in soft agar, there remains little doubt that the cytotoxicity of nitrosoureas both in normal and in neoplastic cells is enhanced by the concurrent administration of CFN and CPZ, despite differences in DNA repair enzyme content among human and rodent cells.

The failure to observe such enhancement in a clinical trial either by increase in tumor response or in degree of toxicity may have several explanations.

The actual in situ drug levels may differ among mice and patients, despite the similarity of doses expressed in milligrams per meter squared for CFN and CPZ. The relevance of advanced preclinical tumor models may be further improved by comparing actual plasma levels of drugs in mice and humans.

Clinical toxicity data are not easily amenable to statistical analysis, since (unlike in preclinical experimentation) in the majority of patients tumor progression precludes further drug administration, and ethical restrictions call for dose adjustments.

Finally, although a large number of patients were entered into each treatment arm, the events of interest, namely, partial or complete remissions and episodes of severe myelosuppression, were too rare to allow a conclusion as to the efficacy of the combination.

The xenograft model offers a partial solution to the last problem by using a human melanoma with defined sensitivity to nitrosoureas in each treatment group.

While the enhancement of cytotoxicity has been observed for cyclophosphamide, melphalan (15), and cisplatin (16), we have observed no enhancement of cytotoxicity from cisplatin, ifosfamide, or dacarbazine, all of which were very active against this particular human melanoma xenograft line (R. Osieka, unpublished observations). This may be an indication that the

mechanism whereby drug potentiation occurs is not operative in every individual tumor. This concurs with the observation by Rose et al (1), who found a different degree of enhancement in various rodent transplantation tumors.

The testing of hypotheses derived from preclinical data by conducting randomized clinical trials may lead to errors. The failure to confirm a hypothesis may not be due to the irrelevance of murine experimental systems but to the limited power of certain clinical trials to detect differences in response.

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Carboplatin, An Active Drug in Advanced Head and Neck Cancer[1]

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Because of the clinical activity of carboplatin in several types of tumors, this drug was studied in a phase II trial of 25 patients with advanced head and neck cancer [stage IV (M_0)] without prior treatment. Six patients (24%) achieved objective response, including two patients with complete response. Carboplatin toxicity was mild in most patients, with nausea, vomiting, and myelosuppression being the most frequent side effects. No renal or neurologic toxicity was observed. Further trials of carboplatin in advanced head and neck cancer are warranted. [Cancer Treat Rep 70:1173-1176, 1986]

In the last decade significant advances in chemotherapy for head and neck cancer have been made. It is no longer considered only palliative therapy for locoregional or metastasized disease but rather an integral part of a combined treatment approach. In this setting chemotherapy is used as an induction therapy which permits a second curative procedure with surgery and/or radiotherapy (1); nonetheless, the survival rate at 2 years is only around 30% in advanced head and neck cancer (2). Cisplatin appears to be one of the most effective chemotherapeutic agents, whether used alone or in a polychemotherapeutic regimen; however, because of substantial toxicity it is usually administered on an inpatient basis.

Carboplatin is a second-generation platinum analog with reduced incidence and severity of renal and neurological toxicity in comparison to its analog cisplatin. Antitumoral activity in ovarian cancer (3) and small cell lung cancer (4) has been reported. In phase I trials using a 400-500-mg/m^2 single iv dose, reversible myelosuppression, particularly thrombocytopenia, was the dose-limiting toxic effect (5). The following phase II trial was carried out to search for a more effective and less toxic chemotherapeutic induction drug in advanced head and neck cancer.

MATERIALS AND METHODS

Between December 1984 and August 1985, 25 consecutive patients with advanced head and neck cancer were entered in a phase II trial. Eligibility criteria included: histological proof of squamous carcinoma in the oral cavity, pharynx, or larynx; age $\leq$ 70 years; Karnofsky performance status $>$ 60%; stage IV (M_0) in the UICC (International Union Against Cancer) classification; no prior treatment; serum creatinine $<$ 130 μmols/L; serum albumin $>$ 30 g/L; hemoglobin $>$ 9 g/dl; wbc count $>$ 4000/mm^3; and platelet count $>$ 150,000/mm^3. Informed consent was obtained from all patients.

Characteristics of the 25 patients entered in this study are shown in table 1.

Carboplatin (supplied by the Investigation Department of Laboratorios Ferrer, Barcelona, Spain) at a dose of 400 mg/m^2 was given in 500 ml of 5% dextrose in water as a 1-hour iv infusion every 28 days. A diagram of the trial is shown in figure 1, where standard therapy consists of three cycles of induction chemotherapy using bleomycin, methotrexate, and cisplatin (BMP), as described by Vogl et al (6), followed by surgery and/or radiotherapy depending on the tumor site.

Pretreatment study parameters included a history and physical examination, cbc and platelet count, liver and renal function tests (serum urea and creatinine), serum albumin, and chest x-ray. More specific tests such as laryngograms, larynx tomograms, or computerized tomographic scans of the head and neck were performed if considered useful for the pretreatment staging or response evaluation. The head and neck examination was always done by the same physicians.

Before each course of treatment, history and physical examination were taken and renal function tests and cbc and platelet count were performed for response and toxicity evaluation.

Complete response was defined as the disappearance

[1] Received Feb 5, 1986; revised May 28, 1986; accepted June 9, 1986.

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[3] We thank the following investigators who entered patients in the study: Drs. J. Burgues and M. Vega from the Hospital de la Santa Creu i Sant Pau. We also thank Mrs. R. Milla for her help in preparing this manuscript.

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TABLE 1.—Patient characteristics and response

Patient No.	Age (yrs)	Karnofsky performance scale (%)	Primary site	Tumor grade	Stage (UICC)	Response*
1	70	90	Larynx	I	$T_4N_0M_0$	PR
2	66	80	Oral cavity	—	$T_4N_0M_0$	F
3	58	70	Larynx	III	$T_4N_3M_0$	PR
4	46	100	Oropharynx	II	$T_3N_3M_0$	SD
5	66	80	Oropharynx	I	$T_4N_0M_0$	F
6	53	80	Oropharynx	II	$T_4N_2M_0$	F
7	47	80	Larynx	II	$T_4N_0M_0$	F
8	65	80	Larynx	II	$T_4N_1M_0$	SD
9	44	80	Larynx	III	$T_4N_1M_0$	CR
10	56	80	Hypopharynx	III	$T_4N_1M_0$	SD
11	69	80	Oropharynx	II	$T_4N_1M_0$	SD
12	66	80	Oropharynx	—	$T_4N_0M_0$	SD
13	53	70	Oropharynx	II	$T_4N_3M_0$	SD
14	52	80	Oral cavity	—	$T_3N_3M_0$	F
15	53	80	Oropharynx	III	$T_4N_0M_0$	CR
16	59	80	Oral cavity	—	$T_4N_0M_0$	F
17	55	90	Hypopharynx	III	$T_4N_1M_0$	PR
18	61	70	Oropharynx	—	$T_4N_1M_0$	SD
19	57	80	Hypopharynx	III	$T_4N_3M_0$	F
20	65	80	Hypopharynx	III	$T_4N_1M_0$	PR
21	54	90	Oral cavity	II	$T_4N_3M_0$	SD
22	38	90	Larynx	I	$T_4N_2M_0$	SD
23	61	80	Larynx	—	$T_2N_2M_0$	SD
24	50	80	Oral cavity	—	$T_4N_3M_0$	SD
25	52	70	Oral cavity	I	$T_4N_1M_0$	SD

*CR = complete response; PR = partial response; SD = stable disease; and F = failure.

of all clinical evidence of tumor. Partial response was defined as a 50% decrease in the sum of the products of the perpendicular diameters of measured lesions without an increase in the size of any lesions or the appearance of new lesions. Stable disease was defined as a $< 50\%$ decrease or a 25% increase in the sum of the products of the perpendicular diameters of measured lesions. Disease progression was defined as a 25% increase of any measurable lesion or the appearance of new lesions. Toxicity was reported according to the World Health Organization classification for acute and subacute toxicity (7).

The double-sampling design developed by Gehan (8) for the study of new drugs was employed.

RESULTS

All of the patients were evaluable for response and toxicity. Fifty-two courses of carboplatin were administered. Six patients received three courses, 15 received two courses, and four received one course. The overall response rate was 24% (95% confidence limits, 4%–44%). Two patients achieved complete response. One patient had a $T_4N_1M_0$ of the larynx and the other (the only woman in the group) had a $T_4N_0M_0$ at the base of the tongue. The complete responses were achieved after the second and third course of carboplatin, respectively.

The patient with a $T_4N_1M_0$ of the larynx had an enlarged neck lymph node (5×4 cm in diameter). After chemotherapy, a laryngopharyngectomy and radical neck dissection confirmed the absence of microscopic tumor in the node; however, a carcinoma in situ was detected in the laryngopharyngectomy section. In the other patient with a $T_4N_0M_0$ at the base of the tongue, external irradiation of the oropharynx followed by an interstitial implant with ^{192}Ir was done; biopsies obtained at the time of the implant were negative. In addition, four patients achieved partial response, all after the first course of carboplatin. Stable disease was observed in 12 patients. The remaining seven patients continued to have disease progression (table 1). After carboplatin, 15 patients received second-line BMP chemo-

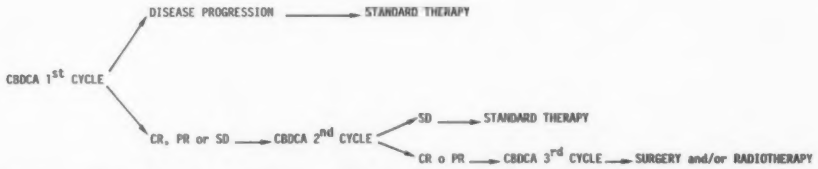

FIGURE 1.—Diagram of the trial. CBDCA = carboplatin; and standard therapy = BPM followed by surgery and/or radiotherapy.

TABLE 2.—Carboplatin toxicity (25 patients)

Toxic effect	No. of patients (%)
Nephrotoxicity	0 (0)
Clinical neurotoxicity	0 (0)
Nausea and vomiting*	15 (60)
Grade I	1
Grade II	12
Grade III	2
Grade IV	0
Hematologic*	8 (32)
Grade I	6
Grade II	1
Grade III	0
Grade IV	1

*WHO classification.

therapy. In two of them there was severe renal and hematological toxicity, with one treatment-related death. Only partial response was achieved in three patients.

The mean follow-up in April 1986 is 9 months (range, 2–16). At that time, six patients are alive without disease, 12 are alive with disease, and seven are dead.

In general, carboplatin showed low toxicity and was well-tolerated in most patients (table 2). Nephrotoxicity or clinical neurologic toxicity was not observed in any patient. Fifteen patients had nausea and vomiting, which usually corresponded to grade I–II of the World Health Organization classification. No antiemetic prophylactic therapy was used in any patient in the first course. One patient had nausea, 12 had less than four emetic episodes, and two had more than six emetic episodes. This last group received prophylactic antiemetic therapy with metoclopramide in subsequent courses.

Hematologic toxicity was observed in eight patients, seven of whom had asymptomatic mild anemia. One patient had granulocytopenia (grade II) after the first cycle of carboplatin and severe hematologic toxicity (grade IV) following the third cycle. During this last episode the patient experienced severe granulocytopenia with fever, low platelet count (41,000/mm^3), and bleeding which required support measures. Leukopenia was not observed in any other patient. No treatment-related deaths occurred. Hematologic toxicity after each course is shown in table 3.

DISCUSSION

Cisplatin-based induction chemotherapy regimens have generally proven to be the most effective in the treatment of advanced head and neck cancer, with an overall response rate > 50% (6,9). There are few studies of induction chemotherapy which have used cisplatin as a single agent. When cisplatin is administered alone, the response rate ranges between 27% and 40% (10,11) and a broad spectrum of toxicity is observed (severe nausea and vomiting and renal, neurological, and mild hematological toxicity) (12).

The results of this study suggest that carboplatin is a moderately active drug in patients with head and neck cancer. Carboplatin yields a response rate of 24% (95% confidence limits, 4%–44%) and therefore its activity looks similar to that of cisplatin. Nevertheless, carboplatin showed a different toxicity spectrum than cisplatin. In our study no patients had renal or neurological toxicity. Nausea and vomiting were the most frequent forms of toxicity which generally fell within grades I–II of the WHO classification. Given that antiemetic prophylactic therapy was not utilized, carboplatin appears to be a moderately emetic drug which could probably be controlled using standard antiemetic treatment. Even though 32% of the patients had myelosuppression it was always mild, except in one patient (4%) in whom it was life-threatening.

In conclusion, this study suggests that carboplatin may be a useful drug in untreated advanced head and neck cancer, with a similar response rate to cisplatin, and showing a toxicity spectrum that is well-tolerated and different from that of cisplatin. These results warrant further study of carboplatin in phase II trials.

TABLE 3.—Hematologic toxicity of carboplatin (25 patients)

	Toxic effect								
	Anemia after Course—			Leukopenia after Course—			Thrombocytopenia after Course—		
Grade*	1	2	3	1	2	3	1	2	3
0	25	15	3	24	21	5	25	21	5
I	0	5	2	0	0	0	0	0	0
II	0	0	1	1	0	0	0	0	0
III	0	1	0	0	0	0	0	0	0
IV	0	0	0	0	0	1	0	0	1
Total	25	21	6	25	21	6	25	21	6

*WHO classification.

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Induction of Doxorubicin Resistance in Heterogeneous Human Colon Tumor Cells by *N*-Methylformamide[1,2]

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Two clonal subpopulations of cells (termed A and D) obtained originally from a heterogeneous human colon adenocarcinoma were studied in vitro with regard to possible modification of responsivity to graded doses of doxorubicin (ADM) after long-term growth in medium containing the differentiating agent *N*-methylformamide (NMF). Non-NMF-adapted clone A and D cells exhibited biphasic response curves after exposure to graded doses of ADM (0–1.4 μg/ml, 1 hour, 37°C). The inactivation slopes for the initial regions of the survival curves were 0.13 and 0.39 g/ml for clone A and D cells, respectively. NMF-adapted cells, however, exhibited decreased sensitivity to ADM killing as the inactivation slopes for the initial region of the survival curves increased to 0.26 and 0.59 g/ml for clone A and D cells, respectively. The final slopes of the biphasic response curves (doses above about 0.8 μg/ml) were not significantly different between clone A and clone D tumor cells in either the non-NMF- or NMF-treated conditions. These data on ADM responses after differentiation induction by NMF indicate that the combination of differentiation-inducing agents with certain chemotherapeutic agents may produce therapeutically undesirable effects and that preclinical data are necessary prior to implementation of possible combined-modality protocols.
[Cancer Treat Rep 70:1177–1180, 1986]

In the past several years we have been studying the biology of tumor cells treated with chemical agents termed polar solvents [ie, *N*-methylformamide (NMF) and *N*, *N*-dimethylformamide (DMF)]. The rationale for this research stems from the fact that exposure of mammalian cells to these agents induces a state of differentiation (1,2). For example, treatment of human tumor cells with NMF or DMF produces changes in cell morphology, decreased clonogenicity in soft agar or as monolayer cultures in vitro, decreased tumorigenicity in vivo, altered antigenicity, and changes in intracellular enzyme profiles and glutathione levels (1–8). Of clinical importance is the finding that such chemically directed phenotypic alterations are also associated with changed sensitivity to certain cytotoxic agents. Specifically, we have shown that both rodent and human tumor cells become more sensitive to the cell killing effects of ionizing radiation, cisplatin, mitomycin, 5-fluorouracil, and bleomycin (9–15). Therefore, we have suggested that the combination of differentiating agents like NMF or DMF with drugs or ionizing radiation may be a productive approach in the strategy of treatment of solid tumors (16–19). This may additionally be of special interest when viewed as a therapeutic strategy for heterogeneous cancers, in which resistance to single agents of tumor cell subpopulations may be one of the limiting factors in curability (11,12,16,20–22), because we have found that polar solvent treatment tends to make the responses of individual subpopulations of heterogeneous tumors more similar to one another for agents such as x-irradiation (11,12,14).

However, we have also noted that exposure of tumor cells to polar solvents may, in some instances, produce no alteration in the phenotypic response to a cytotoxic agent (eg, to ultraviolet light) (J. T. Leith, unpublished data, May 1986), or it may actually induce resistance

[1] Received May 28, 1985; revised May 22, 1986; accepted June 13, 1986.

[2] Supported by American Cancer Society grant PDT-243 and by Public Health Service grants CA-13943 and CA-20892 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. L. A. Ferrari is the recipient of fellowship 007 AIFCR from the American-Italian Foundation for Cancer Research.

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(eg, to hyperthermia) (23). In this communication we show that differentiation induction by NMF may also produce resistance in two subpopulations of human colon cancer cells to an important chemotherapeutic agent, doxorubicin (ADM). This further indicates that if induced cell differentiation is to be a useful route in cancer therapy (19,24), knowledgeable selection of appropriate agents for combination treatment is necessary, particularly as in this instance, NMF is currently in phase I and II trials (25–27).

MATERIALS AND METHODS

Two subpopulations of tumor cells originally cloned from a single heterogeneous human colon adenocarcinoma were used in this study. A complete description of these two subpopulations (clones A and D) and the techniques of isolation has been published (20). In this work, clone A (aneuploid) and D (diploid) cells were routinely maintained as exponential cultures in RPMI-1640 medium supplemented with 10% fetal bovine serum and penicillin-streptomycin (100 U/ml and 100 μg/ml), Hepes buffer (1 M solution, 10 ml/L), tricine buffer (1 M solution, 10 ml/L), anti-PPLO agent (Tylocine, 60 μg/ml), sodium bicarbonate (7.5% solution, 10 ml/L), and gentamicin solution (4 μg/L) [all reagents from the Grand Island Biological Co (GIBCO), NY]. Cells were grown in a humidified 37°C incubator (National Appliance Co, Portland, OR) under a gas environment of 95% air and 5% CO_2. Cells for these experiments had undergone two passages after rejuvenation from frozen stock before use. For experiments, cells from stock cultures were placed into RPMI-1640 medium containing 1% NMF (vol/vol) (Aldrich Chemical Co, Milwaukee, WI) and were passaged three to five times in NMF-containing medium (approximately 2 weeks of growth in NMF medium). Due to differences in growth rate (non-NMF-treated cells have cell culture doubling times of 24 hours, while 1% NMF-treated cells have doubling times of 48 hours), cultures for experiments were set up at a concentration of 1×10 cells/25-cm flask (Falcon Products, Oxnard, CA) (non-NMF-treated cells) or 2×10 cells/flask (NMF-treated cells) 48 hours prior to use. This seeding technique compensates for the twofold difference found in colony-forming ability between non-NMF- and NMF-treated cells and ensures that any cellular effects due to cell passaging have disappeared. For experiments, the medium was removed from the flasks and replaced with fresh, warmed medium containing varying concentrations of ADM (Farmitalia, Milan, Italy) in the range of 0.00–1.40 μg/ml. The cell monolayers were exposed to ADM at 37°C for 1 hour, at which time the drug-containing medium was removed, the monolayers were washed twice with 0.9% NaCl, and then the cells were removed from the flasks by a 7-minute exposure to 0.03% trypsin-EDTA (GIBCO). The trypsinate was combined with an equal volume of RPMI-1640 medium containing serum, centrifuged (1000 rpm for 7 minutes), resuspended in complete medium (without NMF), counted (Coulter model ZM Coulter Counter, Hialeah, FL), and seeded into 60-mm plastic dishes (Falcon Products, Oxnard, CA) for assessment of clonogenic cell survival. At 10–12 days after seeding, the colonies were washed with 0.9% NaCl and fixed and stained with 0.5% crystal violet in absolute methanol. Colonies containing > 50 cells were then counted for survival curve analysis.

The possibility that growth in NMF-containing medium might have selected for cells with a permanent ADM-resistant phenotype was examined by allowing cells fully adapted to NMF to grow in RPMI-1640 medium without NMF for one passage. The cells were then challenged with graded doses of ADM and survival results were compared to those from cells fully adapted to NMF and from control cells.

RESULTS

The results of these studies are shown in figures 1 and 2, where the responses of clone A (fig 1) or clone D (fig 2) cells are shown for non-NMF-treated and NMF-

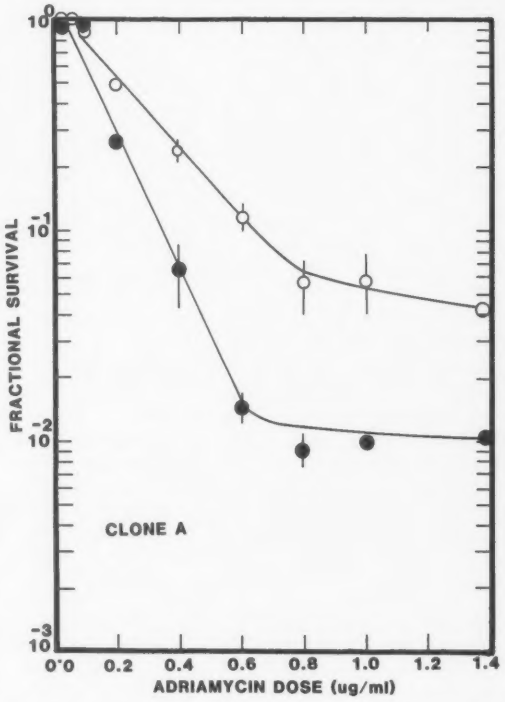

FIGURE 1.—Survival responses of clone A human colon tumor cells after exposure (1 hr, 37°C) to graded doses of ADM. Data are shown for cells grown for 3 passages in medium containing 1% (vol/vol) NMF (○) versus non-NMF-treated control cells (●). Error bars are the standard errors of the mean values.

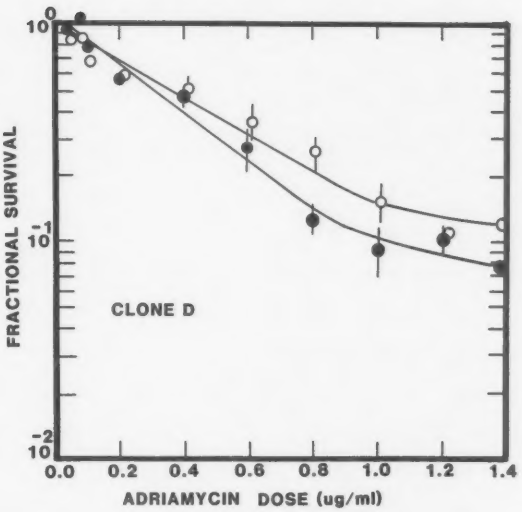

FIGURE 2.—Survival responses of clone D human colon tumor cells after exposure (1 hr, 37°C) to graded doses of ADM. Data are shown for cells grown for 3 passages in medium containing 1% (vol/vol) NMF (○) versus non-NMF-treated control cells (●). Error bars are the standard errors of the mean values.

pretreated cells. The colony-forming efficiencies (CFE) of clone A cells were 43.5% ± 3.24% and 25.0% ± 3.8% (non-NMF- and NMF-treated cells, respectively, mean ± SEM), while similar values for clone D cells were 80.5% ± 7.5% and 50.0% ± 1.3%. Therefore, the NMF treatment reduced the CFE values by about 40% for both cell lines.

The inactivation slopes for the non-NMF- and NMF-treated clone A and D cells are listed in table 1, with their standard errors (28).

It was also found that the survival responses of clone A and D cells that had been allowed to reverse from their NMF-induced state for one passage prior to ADM exposure were equivalent to responses of non-NMF-treated cells (data not shown). There is, therefore, no indication that growth of cells in NMF-containing medium had selected a phenotype of permanent ADM resistance.

DISCUSSION

Two major points may be made from these data. First, there is a significant difference between the intrinsic sensitivities of A and D cells to ADM (summarized in table 1), although both tumor cell lines exhibit a biphasic survival response. Comparing the cell killing rate in the initial exponential region, the inactivation slopes vary by a factor of 3, with clone D cells being the more resistant line (table 1). Our values for ADM responsivity are similar to values found by other investigators (29). This finding is simply another manifestation of neoplastic diversity (16,20).

Second, it is clear that for both clone A and clone D cells, pretreatment growth in NMF-containing medium has induced significant resistance to ADM cell killing. For clone A cells, again comparing inactivation slopes in the initial exponential region of cell kill, the increase in resistance is a factor of about 2, while for clone D cells it is a factor of about 1.5 (table 1). The changes seen in these initial slope values are statistically significant at the 5% level of significance (29). There are no significant differences among any of the plateau region inactivation slope values, although these are all clearly significantly greater than their respective exponential region slope values (by a factor of about 25–30 for clone A cells and about 5 for clone D cells).

We feel that this finding of induced ADM resistance is important, because it has obvious implications about the use of polar solvents such as NMF in cancer treatment (26): ie, there may be situations in which the combination of NMF with certain chemotherapeutic agents (eg, ADM) may be contraindicated.

It is somewhat surprising that NMF treatment has produced apparent ADM resistance, as it has been shown definitively that NMF treatment significantly reduces intracellular glutathione levels (7,8). Such glutathione depletion might then produce increased sensitivity to ADM-induced damage (30). However, as NMF appears to exert its glutathione-depleting action through formation of a conjugate (7,8), and as Babson et al (31) have demonstrated that actual inhibition of glutathione reductase is necessary for the observation of increased ADM toxicity (which NMF does not produce), this observed resistance may be a result of other NMF cellular effects. We have noted that polar solvent treatment is associated with a significantly increased cell volume (32), decreased sensitivity to heat killing (23), and decreased membrane fluidity (D. L. Dexter, personal communication, 1986). All of these data implicate the membrane in NMF-induced responses. Since Siegfried et al (33) and Rintoul and Center (34) have shown an association between decreased membrane fluidity and ADM

TABLE 1.—Effects of NMF on the survival responses of 2 human colon adenocarcinoma subpopulations to ADM treatment

Tumor subpopulation	ADM exposure conditions (D_0)*	
	−NMF	+NMF
Clone A		
Exponential region	0.129 ± 0.008	0.262 ± 0.045
Plateau region	6.13 ± 5.47	2.58 ± 3.07
Clone D		
Exponential region	0.394 ± 0.026	0.588 ± 0.030
Plateau region	2.60 ± 4.59	2.05 ± 3.22

*The D_0 value (μg/ml) is the ADM dose needed to reduce incident survival to 1/e of that level in the linear portion of the first exponential survival region. Inactivation slopes are shown with their standard errors.

resistance, it may be that the observed resistance is mediated through membrane alterations, which may then produce altered drug transport into the cell and/or enhancement of drug efflux mechanisms (35,36). This possibility is supported by the work of Tritton and Yee (37), who bound ADM to the surface of agarose beads and found that the immobilized drug could still produce cell killing of L1210 cells, indicating that membrane contact only was a sufficient condition for toxicity.

In summary, we have demonstrated in vitro that differentiation induction of human cancer cells by NMF may be accompanied by a marked decrease in the response to ADM. However, as such changes in the response phenotype of the tumor cell may be quite different depending on the cytotoxic agent used, further preclinical work on such pleiotropic effects in vitro and in vivo is clearly indicated.

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Doxorubicin Versus Mitomycin Versus Doxorubicin Plus Mitomycin in Advanced Breast Cancer: A Randomized Study[1]

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In a randomized trial the antineoplastic and toxic effects of doxorubicin (ADR), mitomycin (MMC), and the combination of the two were evaluated in postmenopausal patients with advanced breast cancer using the following treatment regimens: (*a*) ADR (75 mg/m^2 by iv bolus every 3 weeks); (*b*) MMC (20 mg/m^2 by iv bolus every 6 weeks); and (*c*) ADR (45 mg/m^2 by iv bolus every 3 weeks) and MMC (10 mg/m^2 by iv bolus every 6 weeks). One hundred one patients were entered in the study. Entrance to single-agent MMC therapy was stopped after allocation of 12 patients because of unacceptable side effects, especially nausea and vomiting, and the suggestion of minor efficacy. One of these patients had partial response, eight had no change, and three had progressive disease. The patients in the ADR and ADR plus MMC group were similar as to the following: age (median, 60 years); menopausal age; disease-free interval; performance status, extent of previous cytotoxic therapy (approximately 90% were pretreated) and radiation therapy; and dominant site of disease but with significantly more involved organ sites in the ADR plus MMC group. Among evaluable patients (42 in the ADR group and 39 in the ADR plus MMC group), response rates were as follows: *complete response*—21 versus five; *partial response*—26 versus 44; *no change*—40 versus 38; and *progressive disease*—12 versus 13 ($P > 0.10$). Median times to disease progression were 5.2 and 7.8 months, respectively (log-rank test, $P = 0.03$), but survival times were similar, 9.3 and 10.2 months, respectively (log-rank test, $P > 0.40$). For the two treatment groups suppression of wbc count was similar, while anemia, thrombopenia, and nausea and vomiting were significantly more common among the ADR plus MMC-treated group. Five treatment-induced deaths were observed in the ADR plus MMC group (one from sepsis; two from diffuse hemorrhage; and two from cardiomyopathy), compared to none in the ADR group. In conclusion, this study disclosed no major advantage of the combination of ADR plus MMC compared to ADR alone as second-line treatment of advanced breast cancer, but results from other studies may imply a possible role of MMC as part of second-line combination chemotherapy regimens. [Cancer Treat Rep 70:1181-1186, 1986]

Doxorubicin (ADR) is recognized as the most efficient single drug in the treatment of advanced breast cancer. Response rates of 50% and 35% are noted among patients who were not previously treated with cytotoxic drugs or were refractory to previous treatment, respectively (1). When combined with other drugs, particularly cyclophosphamide and 5-fluorouracil, response rates of 60% and 40% were observed (1). The response and survival times were, however, of short duration (median, 9 and 16 months), and therefore many new drugs were tested in combination with ADR (1).

One of these drugs was mitomycin (MMC), an alkylating agent first isolated from *Streptomyces caepitosus* in 1958. It has proved to be effective in the intravesical treatment of superficial bladder cancer and in combination chemotherapy for gastric and pancreatic carcinomas (2). MMC does not seem to be superior to ADR when evaluated in phase II trials as single-agent therapy for breast cancer (3,4). Phase II trials using ADR plus MMC mainly in previously untreated patients have yielded response rates of 44%–60%, with median durations of remission of 6–12.5 months (5–8), but the ADR plus MMC combination has not until now been compared with the effect of ADR and MMC as single agents in a randomized fashion. The aim of the present randomized trial was to compare the effect of ADR and

[1] Received Sept 25, 1985; revised May 28, 1986; accepted June 13, 1986.

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MMC alone to the combination of ADR plus MMC in mainly systemically pretreated postmenopausal patients with advanced breast cancer.

MATERIALS AND METHODS

Consecutive patients referred to the Department of Oncology from January 1981 to July 1984 were considered for the trial. Criteria of eligibility were (*a*) histological evidence of breast cancer; (*b*) progressive disease with measurable and/or evaluable lesions according to the International Union Against Cancer (UICC) criteria (9) (lymphedema, hilar enlargement, pleural effusion, ascites, bone marrow suppression, and osteoblastic skeletal lesions were not considered as evaluable lesions); (*c*) performance status < 2 (10); and (*d*) age < 70 years. Criteria of ineligibility were (*a*) previous treatment with anthracyclines; (*b*) wbc count $< 3000/mm^3$ and/or platelet count $< 100{,}000/mm^3$; (*c*) evidence of renal (creatinine > 1.2 mg/dl) or hepatic (bilirubin > 1.5 mg/dl) dysfunction; (*d*) CNS metastases; (*e*) congestive heart failure, significant arrhythmia, bilateral bundle branch block, or history of myocardial infarction; (*f*) previous or concurrent malignancies at other sites with the exception of adequately treated carcinoma in situ of the cervix uteri and basal or squamous cell carcinoma of the skin; and (*g*) previous systemic antineoplastic treatment < 4 weeks prior to the trial.

Trial Design

Patients were centrally registered and allocated randomly in a nonstratified way to treatment with ADR, MMC, or ADR plus MMC. The therapeutic regimens were as follows: ADR (75 mg/m^2 by iv bolus every 3 weeks); MMC (20 mg/m^2 by iv bolus every 6 weeks); and ADR (45 mg/m^2 by iv bolus every 3 weeks) and MMC (10 mg/m^2 by iv bolus every 6 weeks).

Dose Modifications

Drug administration was postponed for 1 week if the platelet count ($\times 10^3/mm^3$) was < 100 or the wbc count ($\times 10^3/mm^3$) was < 3. Otherwise, the drug dose was adjusted according to the following hematological values: platelet count > 100 and wbc count > 3, 100% dose; platelet count 50–99 or wbc count 2.0–2.9, 50% dose; and platelet count < 50 or wbc count < 2, no drug.

Treatment Duration

Treatment was to be given for a minimum of 6 weeks and to continue until disease progression or until a cumulated ADR dose of 550 mg/m^2 was reached. Patients having received ADR as single agent were thereafter observed without treatment, while patients receiving ADR plus MMC continued treatment with MMC at an escalated dose. No patients in the ADR plus MMC group reached the maximal ADR dose.

Pretreatment and Follow-up Investigations

Prior to entry in the study a physical examination was done, including assessment of performance status and evaluable lesions, blood cell counts (hemoglobin, wbc, and platelet), clinical chemical tests (serum creatinine, serum calcium, serum alkaline phosphatase, serum transaminase, and serum bilirubin), ECG and echocardiogram or multigated analysis scan, and, if appropriate, ultrasonography of the liver and photograph of cutaneous lesions. During treatment these examinations were repeated every 3–6 weeks and after treatment was stopped approximately every 3 months until disease progression. The patients then usually received endocrine therapy with physical and paraclinical examinations at regular intervals. Exact death data were supplied by the national population register of Denmark.

Definition of Response

Patients were evaluated for response 6 weeks after initiation of therapy and then every 6 weeks. Assessment of response, time to treatment failure, and duration of survival were defined according to UICC criteria (9).

Patients with early progressive disease or early death (ie, before 6 weeks) were registered as having progressive disease.

No extramural review has been made, but x-rays have been reviewed by the investigators supplementary to the descriptions given by the department of radiology. Side effects were graded according to World Health Organization (WHO) criteria (10).

Statistical Methods and Goals

Fisher's exact test or chi-square test was used for comparison of binominal data. Ranked data were compared using the Wilcoxon rank-sum test for unpaired data. Confidence limits for difference in response rates were calculated according to Wulff (11). Survival time and time to disease progression were analyzed by Kaplan-Meier estimates and confidence limits calculated according to Peto et al (12). Data from each treatment group were compared by the log-rank test.

All analyses were performed as two-sided significance tests. Based on an expected response rate of 60%, a total of approximately 50 patients in each treatment group were required to disclose a 25% difference in response rate in pair-wise comparisons of the three groups ($2\alpha = 0.10$; $\beta = 0.20$). If no responses were observed among the first 14 patients in a group, entrance should be stopped, since this would imply a response rate $< 25\%$.

RESULTS

From January 1981 to July 1984, a total of 101 patients were entered in the trial. Until December 1981, a total of 12 patients were randomized to treatment with MMC. In that early stage of the trial it was the clinical impression that the efficacy of MMC alone was inferior

TABLE 1.—Patient characteristics*

Characteristic	ADR ($n = 42$)	ADR + MMC ($n = 39$)
Median age in yrs (range)	59 (46–67)	61 (44–70)
Menopause (yrs)		
0–1	2	3
2–5	9	7
6+	31	29
Disease-free interval (yrs)		
0	7	5
1–2	20	15
3+	15	19
Performance status		
0	10	25
1	26	12
2	6	2
Dominant site of disease		
Viscera	9	15
Bone	15	12
Soft tissue	18	12
No. of organ sites involved†		
1	22	9
2	15	20
3	4	8‡
4	1	2
5	0	0
6	0	0
Radiation therapy for primary disease and lymph nodes	32	29
Radiation therapy for metastatic disease	20	20
No prior radiation therapy	6	4
Adjuvant chemotherapy	7	11
No. of regimens for metastatic disease		
1	31	27
2	1	1
No prior chemotherapy	4	3

*Unless otherwise specified, values = No. of patients.
†Lung, liver, bones, nodes, skin, and others.
‡$P = 0.01$.

to the other two regimens and that the toxicity related to that treatment was intolerable. An interim analysis of the 12 patients demonstrated progressive disease in three patients, no change in eight, and partial response in one. The response later appeared to last for 23 months. Four patients had grade IV nausea and vomiting, two had grade III, three had grade II, and three had grade I. Four patients had long-lasting grade III–IV thrombopenia, with minor bleeding, and one had extravasation which caused necrosis necessitating skin graft. Consequently, the entrance to that treatment regimen was stopped despite the fact that the statistical requirement for early stoppage was not met.

Accordingly, the following relates only to patients allocated to the other two treatment regimens. Forty-five patients were allocated to receive treatment with ADR and 44 were to receive treatment with ADR plus MMC. Four patients were ineligible because of lack of evaluable lesions (two patients), leukopenia (one), and performance status > 2 (one). Forty-two patients treated with ADR were readily evaluable (four of these patients died or had progressive disease preventing further treatment within 6 weeks). In the group receiving the combination therapy, 39 patients were evaluable (two patients died within 6 weeks of treatment). Two patients in each group were not evaluable for response because of early treatment refusal, but they are included in the analyses of time to disease progression, survival, and toxicity. No patients were lost to follow-up. The two groups were comparable in all aspects but one, since the number of organ sites involved was significantly higher in the ADR plus MMC-treated group (table 1). No differences were noted as to the extent of previous radiation therapy and chemotherapy or response to the latter. It is noted that 38 of 42 patients in the ADR group and 36 of 39 in the ADR plus MMC group had received prior chemotherapy; five of 42 and six of 39 had been treated only with adjuvant therapy.

The overall response rates (complete + partial) were similar in the two groups (47% and 49%, respectively) (table 2). The difference in response rate was (95% confidence limits ± 22%) in favor of ADR plus MMC.

Analysis of the response in relation to dominant site of disease revealed no significant differences between the two treatment regimens. No difference between the groups was noted for treatment response in relation to the extent of previous cytotoxic treatment. In the actual treatment response to prior cytotoxic therapy for advanced disease there were no differences between the two groups, and no trend was noted in either group towards a higher response rate among patients who had also responded to previous treatment.

The overall time to disease progression (median, 95% confidence limits) for ADR versus ADR plus MMC was 5.2 months (range, 4.7–6.5) and 7.7 months (range, 5.4–10.1), respectively, with significant difference ($P < 0.05$) at log-rank test in favor of ADR plus MMC (fig 1). The time to progression for responders (complete response + partial response) was 6.7 months (range, 5.7–8.8) and 10.0 months (range, 6.1–11.7), with significant difference ($P < 0.02$) at log-rank test. The overall survival was 9.3 months (range, 7.2–13.4) and 10.2 months (range, 9.0–16.7), log-rank test, $P > 0.40$. At 30 months the difference in survival was 8% (95% confidence limits,

TABLE 2.—Response in 81 evaluable patients*

Response	ADR n (%)	ADR + MMC n (%)
Progressive disease	5 (12)	5 (13)
No change	17 (40)	15 (38)
Partial	11 (26)	17 (44)
Complete	9 (21)	2 (5)
Complete + partial	20 (47)	19 (49)

*$P > 0.10$ for all comparisons.

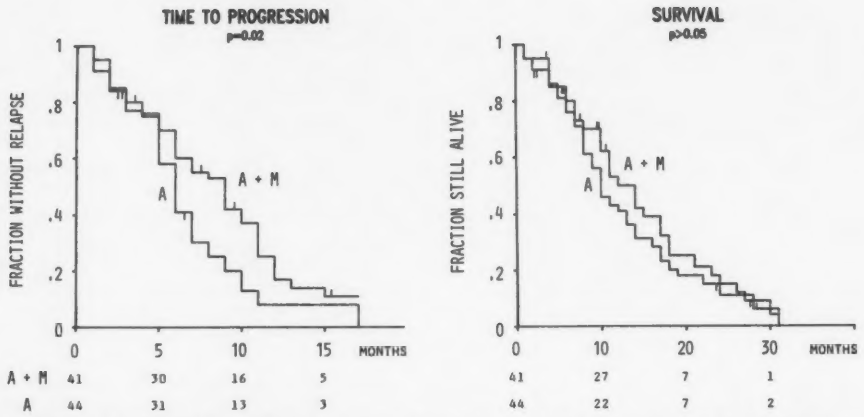

FIGURE 1.—ADR (A) vs ADR + MMC (A+ M). Time to disease progression and duration of survival. Vertical bars indicate censored patients, ie, patients still not relapsed or patients still alive. No. of patients at risk is given below the abscissa.

-5% to 21%) (fig 1). The survival for responders was 12.1 months (range, 8.4-16.3) and 16.4 months (range, 10.2-27.3), log-rank test, $P > 0.20$). At the time of analysis six and eight patients were still alive, respectively, two and four having had no relapse.

The evaluation of the hematological toxicity is indicated in table 3. The two treatment groups had similar pretreatment values of hemoglobin and wbc and platelet counts. The suppression of wbc counts was also of equal size with median nadirs of 2.6 versus 2.0 ($\times 10^3$/mm^3). A fall in hemoglobin was noted in both groups, but the mean and minimum values were significantly lower in the ADR plus MMC-treated group. A highly significant difference was observed in the platelet counts with a huge suppression in the ADR plus MMC group, while the mean value rose in the ADR group. Two patients in each group received treatment for sepsis, with a fatal outcome for one patient in the ADR plus MMC group. Two other patients in this group died from diffuse hemorrhage due to prolonged thrombopenia. Occurrences of nausea and vomiting are shown in table 4. A statistically significant ($P = 0.03$) increased frequency of intolerable nausea and vomiting is noted for patients treated with ADR plus MMC. Five patients treated with ADR plus MMC and three treated with ADR refused prolonged treatment because of subjective gastrointestinal discomfort. Fifteen versus 14 patients in the ADR and ADR plus MMC group had stomatitis, predominantly grade I-II. Almost all patients experienced grade II-III alopecia. Congestive heart failure of proven or strongly suspected chemotherapy-induced etiology was noted in two patients treated with ADR plus MMC, both of whom died from cardiomyopathy after having received 512 and 415 mg/m^2 of ADR and 45 and 47 mg/m^2 of MMC, respectively, and in one patient treated with 550 mg/m^2 of ADR. This patient responded to treatment with digitalis and diuretics. No obvious cases of MMC-associated hemolytic-uremic syndrome were observed. However, five MMC-treated patients had episodes of severe dyspnea, often after having received blood transfusions, and usually responding to steroids, diuretics, and digitalis.

TABLE 3.—Hematological toxicity among eligible patients*

Blood cell counts	ADR	ADR + MMC
Hemoglobin (mmols/L)		
Start	7.9 (5.9-9.6)	7.8 (6.0-9.6)
Mean	7.1 (5.8-8.4)	6.6 (5.4-9.6)†
Nadir	6.4 (4.9-8.3)	5.5 (2.5-8.2)†
Wbc count ($\times 10^3$ cells/mm^3)		
Start	5.3 (5.3-10.3)	5.4 (3.0-9.6)
Mean	3.9 (2.6-8.2)	3.6 (2.3-8.3)
Nadir	2.6 (1.6-7.0)	2.0 (0.3-7.8)
Platelet count ($\times 10^3$ cells/mm^3)		
Start	268 (120-992)	262 (128-534)
Mean	328 (164-720)	146 (91-386)‡
Nadir	246 (84-615)	90 (46-130)‡

*Median values (range) are given for (*a*) pretreatment measurements, (*b*) mean values for each patient based on all hematologic tests during the treatment period, and (*c*) nadir recorded during the treatment.
†$P < 0.005$.
‡$P < 0.00001$.

TABLE 4.—Nausea and vomiting among eligible patients*

Grade	ADR	ADR + MMC
0	0	3
1	15	6
2	21	12
3	6	15
4	2	5
Total	44	41

*$0.025 < P < 0.05$. Maximal toxicity recorded.

The fraction of the theoretically maximal cumulated amount of drug during the treatment period which was actually administered is calculated for the two regimens. In the ADR group 78% and in the ADR plus MMC group 66% of the ADR and 71% of the MMC dose was received, indicating that dose modifications and treatment delays for all reasons were more common in the ADR plus MMC group.

DISCUSSION

Despite incontestable progress during the last decades in the development of effective combination chemotherapy regimens for advanced breast cancer, the systemic cytotoxic and endocrine treatment remains palliative. The goal of ongoing research is to introduce new and more effective drugs as well as regimens with at least the same activity as now, but with less toxicity.

The purpose of the present study was to evaluate whether the combination of ADR plus MMC was superior to single-agent therapy with ADR or MMC with respect to antineoplastic effect and toxicity. While this study failed to compare ADR versus MMC as single agents in patients previously untreated with anthracyclines, it is the impression from the literature that MMC is not superior to ADR but may represent a second-line alternative among patients refractory to ADR in ADR-containing combinations (3,4,8,13,14). Prior to this study the combination of ADR plus MMC has been evaluated only in phase II trials, with treatment results encouraging further studies. This randomized study, however, has not been able to prove any difference between the response rates achieved with ADR alone and in combination with MMC. A small but significant improvement of the time to disease progression was noted in favor of ADR plus MMC, but no differences in survival were observed. The observed response rates of 47% and 49% are similar to the results obtained in other studies of second-line combination chemotherapy. The overall time to disease progression (5.2 and 7.7 months) is in accordance to other studies, including an early report from this department [approximately 4 months for second-line treatment with ADR (15)]. The survival time of 9.3 and 10.2 months may seem low, especially when taking into account the relatively good performance status and modest organ involvement of the patients. Nevertheless, it correlates well to a median survival time of 11 months which was reported in a cooperative trial of ADR as second-line chemotherapy to which this department contributed a substantial number of patients (16).

The distribution of the responding patients between complete response and partial response was rather uneven, with surprisingly few complete responders in the ADR plus MMC group. This, however, was not reflected in the outcome of the responding patients, since the median time to disease progression was 6.7 and 10.0 months and the log-rank test showed a significant prolongation of the time to progression in the ADR plus MMC group while the survival time was alike.

The observed hematological toxic effects preclude that lack of therapeutic superiority in the ADR plus MMC group was due to insufficient doses of the administered drugs, since the suppression of the wbc count was equal in the two treatment groups and that of the platelet count was much more pronounced in this group. The subjective side effect in both groups was predominantly nausea and vomiting to an extent which probably exceeds what is commonly experienced with these drugs. This may partially be due to interobserver variations, despite strictly defined criteria for reporting toxic effects, but on the other hand the observed toxic effects are not in conflict with other reports, eg, Amiel et al (8). The gastrointestinal toxic effects were more pronounced in the ADR plus MMC group, which was also the case for the cardiopulmonary toxic effects, since two fatal cases of cardiomyopathy and five cases of pulmonary distress were seen. The high incidence of intractable cardiac toxicity may reflect a possible synergistic effect of the two drugs, which has been proposed earlier (17). A biopsy performed on one of the patients who died from cardiomyopathy showed a histological picture typical for ADR-induced cardiomyopathy. Among the patients treated with ADR plus MMC there were no clear-cut cases of the syndrome of microangiopathic hemolytic anemia with thrombopenia and renal failure which has been described in connection with treatment with MMC (2). The two patients who died of hemorrhage after prolonged thrombopenia and the patients who experienced pulmonary toxicity showed no signs of renal impairment or hemolysis. None of these cases were fatal.

The present study has not been able to indicate superiority of ADR plus MMC compared to ADR alone in the treatment of advanced breast cancer. On the other hand, phase II trials with MMC in other combinations as second- or third-line treatment have shown some encouraging results. Thus, combinations of MMC with ADR plus vinblastine (18,19), ADR plus 5-fluorouracil and vincristine (20), ADR plus mitolactol (21), vinblastine (22), and vinblastine plus mitolactol (23) have yielded response rates of 31%–73%, with toxic effects comparable to those described in the present study. This indicates that combinations with MMC may be an alternative to other regimens as second- or third-line chemotherapy for advanced breast cancer.

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Effect of High-Dose Cyclophosphamide and Total-Body Irradiation on Left Ventricular Function in Adult Patients With Leukemia Undergoing Allogeneic Bone Marrow Transplantation[1,2]

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Protocols used to prepare patients with leukemia for bone marrow transplantation have the potential for cardiac toxicity due to high-dose cyclophosphamide and total-body irradiation. We have reported one regimen, combining cytarabine (5 mg/kg), cyclophosphamide (90 mg/kg), and total-body irradiation (900 cGy), which is relatively effective in the treatment of leukemia. To assess cardiac effects of this treatment regimen, we performed serial echocardiography and radionuclide ventriculography in 28 patients with leukemia (age range, 18–48 years; mean, 31; 21 males) undergoing allogeneic bone marrow transplantation. No significant change in left ventricular fractional minor axis shortening or increase in left ventricular diastolic dimension was seen with weekly echocardiography. At an average of 77 days (range, 28–358) after transplant, repeat radionuclide ventriculography in 17 patients revealed no significant change in resting left ventricular ejection fraction compared to that on admission to the hospital (58% ± 6.8% vs 56% ± 8.0% SD; P = not significant). In seven of these 17 patients (41%), resting ejection fraction fell between baseline and discharge (from mean of 60% to 50%). Resting ejection fraction in four of these patients (23% of the entire group) fell into the abnormal range (from mean of 56% to 44%; lowest, 41%). Ten patients also had exercise radionuclide ventriculography and all had normal responses (> 5% increase with exercise) pre- and post-transplant. We conclude that this effective bone marrow transplantation regimen has little apparent short-term cardiac toxicity in the majority of patients; since a few patients do exhibit a deterioration in left ventricular function, continued cardiac surveillance is probably indicated in posttransplant patients. [Cancer Treat Rep 70:1187–1193, 1986]

High-dose cyclophosphamide and total-body irradiation are frequently used in regimens to prepare patients with leukemia for bone marrow transplantation (1). The cardiac complications of high-voltage radiation therapy have been well-documented (2,3). The cardiac complications of high-dose cyclophosphamide have also been described (4–13), and a recent study would suggest that cardiac toxicity of cyclophosphamide at doses of 180 mg/kg is quite common (6).

We have previously reported a relatively effective regimen to prepare patients with leukemia for bone marrow transplantation that combines cytarabine (5 mg/kg), cyclophosphamide (90 mg/kg), and total-body irradiation (900 cGy) (14). Since radiotherapy and high-dose cyclophosphamide have both been associated with adverse cardiac sequelae, we postulated that the combination of these treatment methods might produce left ventricular dysfunction. To determine the presence and degree of cardiac toxicity associated with our protocol, we prospectively evaluated 28 patients receiving this regimen with serial radionuclide ventriculography and echocardiography.

[1] Received Jan 30, 1986; revised June 9, 1986; accepted June 19, 1986.

[2] Supported in part by Public Health Service grant RR-00059 from the Division of Research Resources, National Institutes of Health (NIH), Department of Health and Human Services (DHHS). D. J. Skorton is the recipient of Research Career Development Award HL-01290 from the National Heart, Lung, and Blood Institute, NIH, DHHS.

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[4] We thank Carolyn Frisbie and Rita Yeggy for their preparation of the manuscript.

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METHODS

Patient Population

All patients > 18 years of age undergoing bone marrow transplantation for leukemia at the University of Iowa were eligible for the study. Of 88 patients undergoing bone marrow transplantation between October 1980 and May 1984, there were 28 patients who were entered in the study; 17 patients completed the study. Eight patients died prior to completion of the study, and three did not complete the study for other reasons. The study protocol and consent forms were approved by the human subjects research review committee of the University of Iowa. Informed consent was obtained from all patients entered. Of the 28 patients who were entered in the study, 11 had acute nonlymphocytic leukemia (ANLL), three had undifferentiated acute leukemia, seven had chronic myelogenous leukemia (CML), six had acute lymphocytic leukemia (ALL), and one had lymphoblastic lymphoma (table 1). Previous chemotherapy and radiation therapy are described in table 1. These 28 patients ranged in age from 18 to 48 years, with a mean age of 31. There were 21 males and seven females. No patient had prior cardiac disease. One patient had a prior history of hypertension, and one had type I diabetes mellitus.

Treatment Protocol

The chemotherapy regimen consisted of cytarabine (5 mg/kg) on Days −7 and −2 (ie, preceding bone marrow transplantation), cyclophosphamide (90 mg/kg) on Day −5, and total-body irradiation (900 cGy at a dose rate of approximately 8 cGy/minute) on Day −1, with bone marrow transplantation on Day 0 (table 2).

Cardiac Diagnostic Methods

The following diagnostic methods were used to monitor the left ventricular size and function of the study patients: cardiovascular history and physical examination, M-mode and two-dimensional (2D) echocardiography, and rest and exercise radionuclide ventriculography.

Echocardiography.—On admission to the hospital, baseline 2D and 2D-derived M-mode echocardiograms were performed using a 2.25- or a 2.4-MHz phased array system. During the period of inhospital isolation (following chemotherapy and irradiation), portable (bedside) M-mode echocardiograms were performed using a 2.25-MHz system. Before discharge, 2D and M-mode studies were repeated. Measurements of left ventricular minor axis at end-diastole and end-systole were made and percent fractional shortening values derived based on standard recommendations of the American Society of Echocardiography (15).

Radionuclide ventriculography.—Equilibrium gated blood pool studies (radionuclide ventriculograms) were performed in all patients at admission and at discharge. In vivo labeling of rbcs was achieved with 25 mCi of iv injected ^{99m}Tc-pertechnetate 20 minutes after the iv administration of 5.1 mg of stannous chloride in the form of stannous pyrophosphate (Mallinckrodt Nuclear). Rest and exercise left ventricular ejection fraction studies in ten patients utilized 30 mCi of ^{99m}Tc pertechnetate for rbc labeling. After gated blood pool images at rest were obtained, each patient performed supine bicycle exercise. The supervising physician determined the maximal work load at which the patient could maintain exercise for the time necessary to acquire exercise gated blood pool images (typically 3–5 minutes at 75 watts). In our laboratory, ejection fractions determined by radionuclide ventriculography have a reproducibility of ± 5% (ie, variation between independent analyses of the same radionuclide study).

All patients had baseline echocardiograms and radionuclide ventriculograms on admission to the hospital (Day −8). Echocardiography was done 1 day after cyclophosphamide (Day −4), 1 day after bone marrow transplantation (Day +1), and weekly thereafter. Because of intercurrent illnesses, some of the weekly echocardiograms were deferred by patient request. All studies were repeated prior to hospital discharge.

Statistical Analysis

The admission (baseline) and repeat (posttransplant) radionuclide ejection fractions were compared using a paired t test. A one-way analysis of variance was used to compare mean weekly echocardiographic measurements.

RESULTS

Table 1 gives the pretreatment characteristics and outcomes of the patients. Four patients died of gram-negative sepsis; one patient each died of fungal sepsis, *Pneumocystis carinii* pneumonia, fulminant graft-versus-host disease, and interstitial pneumonitis.

Clinical Events

The patient dying of fungal sepsis (No. 2) developed tachycardia, an S_4 gallop, and a left ventricular lift. There was no clinical evidence of congestive heart failure in this patient. Patient 17 developed a pericardial rub and pericardial effusion was noted on echocardiography. Other than these patients, no others developed apparent abnormal cardiac signs or symptoms.

Evaluation of Left Ventricular Size and Function

At an average of 77 days (range, 28–358) after transplant, repeat radionuclide ventriculography in the 17 surviving patients who completed the study showed no significant change in mean resting left ventricular ejection fraction compared to the baseline study (58% ± 6.8% versus 56% ± 8.0% SD; P = not significant) (fig 1). In seven of the 17 patients (41%), resting ejection fraction fell between baseline and discharge (from mean of 60% to 50%). These patients could not be distinguished from the remainder of the patients based on previous chemotherapy or on echocardiographic left ventricular dia-

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Sex	Diagnosis	Previous therapy*	Outcome
1	22	F	ANLL	ROAP, doxorubicin, Ara-C, vincristine, AOAP, POMP, amsacrine	Completed study
2	28	F	ANLL (M2)	TADPO	Died on Day 83, fungal sepsis
3	42	M	Acute leukemia	Anthracycline-containing regimen	Completed study
4	22	M	ANLL	Anthracycline-containing regimen, levamisole, methotrexate	Completed study
5	29	M	CML	Busulfan, splenectomy, hydroxyurea	Completed study
6	18	F	ALL	Cyclophosphamide, vincristine, prednisone, daunorubicin, methotrexate, Ara-C, thioguanine	Completed study
7	32	M	ANLL (M1)	TADPO	Died on Day 60, interstitial pneumonitis
8	27	M	ANLL	Anthracycline-containing regimen, Ara-C	Completed study
9	23	M	Acute lymphocytic leukemia	?	Withdrew
10	28	M	ALL	Methotrexate, vincristine, doxorubicin, cyclophosphamide	Withdrew
11	33	M	CML	?	Withdrew
12	45	F	ANLL	Anthracycline regimen, ?, thioguanine, Ara-C	Died on Day 65, sepsis
13	31	M	CML	Busulfan, hydroxyurea	Completed study
14	48	M	ANLL	Daunorubicin, HAT, Ara-C, thioguanine, TAD	Died on Day 8, intracranial hemorrhage
15	20	M	ALL	L-Asparaginase, 6-mercaptopurine, prednisone, vincristine, intrathecal methotrexate	Died on Day 19
16	30	M	Acute undifferentiated leukemia	Daunorubicin, Ara-C, vincristine, intrathecal methotrexate	Died on Day 72, gram-negative sepsis
17	41	M	ANLL (M2)	Ara-C, daunorubicin, TAD, intrathecal methotrexate	Died on Day 64, sepsis
18	32	F	ANLL	Daunorubicin, Ara-C, thioguanine	Completed study
19	40	M	CML	Busulfan, hydroxyurea, splenectomy	Completed study
20	18	M	ALL	?	Died on Day 103, *Pneumocystis carinii* pneumonia
21	41	F	Acute undifferentiated leukemia	TADPO, Ara-C	Completed study
22	28	M	Lymphoblastic lymphoma	1500 rads mediastinum, L-10, COMLA, doxorubicin, vincristine, Ara-C, methotrexate	Completed study
23	28	M	ANLL (M3)	TAD × 2, thioguanine, Ara-C	Completed study
24	37	M	ANLL (M4)	TADPO, thioguanine, Ara-C	Completed study
25	37	F	CML	None	Completed study
26	45	M	CML	Busulfan, hydroxyurea	Completed study
27	23	M	ALL (L1)	HOP-L, intrathecal methotrexate	Completed study
28	24	M	CML	Splenectomy, hydroxyurea	Completed study

*ROAP = rubidizone, vincristine, cytarabine, and prednisone; Ara-C = cytarabine; AOAP = cytarabine, vincristine, L-asparaginase, and prednisone; POMP = 6-mercaptopurine, vincristine, methotrexate, and prednisone or prednisolone; TADPO = thioguanine, cytarabine, daunorubicin, prednisone, and vincristine; HAT = doxorubicin, cytarabine, and thioguanine; TAD = thioguanine, cytarabine, and daunorubicin; L-10 = doxorubicin, vincristine, prednisone, L-asparaginase, cranial radiation therapy (2400 cGy), intrathecal methotrexate, 6-mercaptopurine, dactinomycin, carmustine, methotrexate, and cyclophosphamide; COMLA = cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine; and HOP-L = doxorubicin, vincristine, prednisone, and L-asparaginase.

TABLE 2.—Treatment protocol

Day	Treatment
−8	Admitted to hospital
−7	Cytarabine
−6	
−5	Cyclophosphamide
−4	
−3	
−2	Cytarabine
−1	Total-body irradiation
0	Bone marrow transplantation
1	Hospital isolation; time of discharge
Variable period	

stolic dimensions. Resting ejection fraction in four of these patients (23% of the entire group of 17) fell into the abnormal range (from mean ejection fraction of 56% to 44%; lowest, 41%). Three of four of these patients had had previous anthracycline-containing chemotherapeutic regimens. Ten patients (Nos. 8–17) also had exercise radionuclide ventriculography and all had normal responses (> 5% increase with exercise) pre- and posttransplant.

Data on baseline left ventricular performance of patients who did not complete the study were similar to data on those who completed the study. The eight patients who died before completing the study had a mean resting left ventricular ejection fraction on hospital admission of 58% ± 5.0%. The entire group of 11 patients who did not complete the study (including the eight patients who died and three others who withdrew) had a mean resting baseline ejection fraction of 59% ± 6.0%. This was quite similar to the mean resting baseline ejection fraction of the 17 patients who completed the study: 58% ± 6.8%.

Table 3 and figure 2 show the mean serial echocardiographic measurements obtained in those patients with technically satisfactory echocardiograms. Figure 3 shows individual values for left ventricular diastolic minor axis dimension at baseline and posttransplant in the 12 patients in whom satisfactory studies were obtained. No statistically significant change in mean left ventricular fractional shortening, left ventricular end-diastolic dimensions, or left ventricular end-systolic dimensions was noted throughout the study.

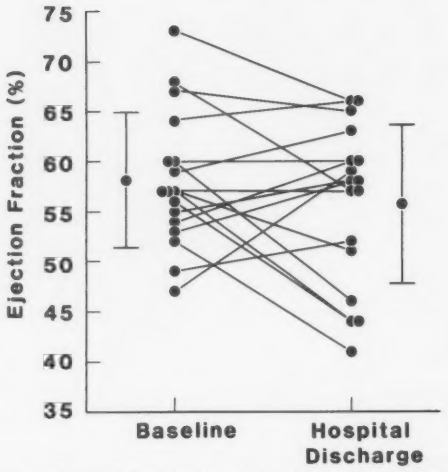

FIGURE 1.—Resting left ventricular ejection fraction, determined using equilibrium gated radionuclide ventriculography, on admission to the hospital and prior to discharge. No significant change in ejection fraction was noted across the entire group of patients. 7 patients showed a decrease in ejection fraction between baseline and discharge; in 4 of these, the ejection fraction fell into the abnormal range.

Pathologic Findings

Postmortem examinations were done in four of the eight patients who died prior to completion of the study; results are noted in table 4. Two of the patients had no cardiac abnormalities. In one patient (No. 2), variable myocardial fibrosis was noted, and in another (Patient 20), bacterial endocarditis was found.

DISCUSSION

The major finding of our study was that, in a group of adult patients with leukemia undergoing bone marrow

TABLE 3.—Echocardiographic data*

	Left ventricular fractional shortening (%)	*n*	Left ventricular diastolic dimension (cm)	*n*	Left ventricular systolic dimension (cm)	*n*
Baseline	36.2 ± 8.4	21	4.9 ± 0.6	21	3.1 ± 0.5	21
After cyclophosphamide	33.6 ± 9.0	18	4.7 ± 0.8	19	3.2 ± 0.5	19
After total-body irradiation	32.3 ± 5.8	15	5.0 ± 0.6	16	3.4 ± 0.4	15
1 wk after BMT†	34.2 ± 5.5	10	5.0 ± 0.7	10	3.3 ± 0.6	10
2 wks after BMT	33.3 ± 10.9	10	5.1 ± 0.9	11	3.4 ± 1.2	11
3 wks after BMT	30.8 ± 7.4	12	4.9 ± 0.5	12	3.4 ± 0.4	12
4 wks after BMT	32.1 ± 6.8	9	4.7 ± 0.6	9	3.2 ± 0.5	9
5 wks after BMT	30.8 ± 8.0	9	5.1 ± 0.7	10	3.5 ± 0.7	10

*Values = mean ± SD; *n* = No. of patients.
†BMT = bone marrow transplantation.

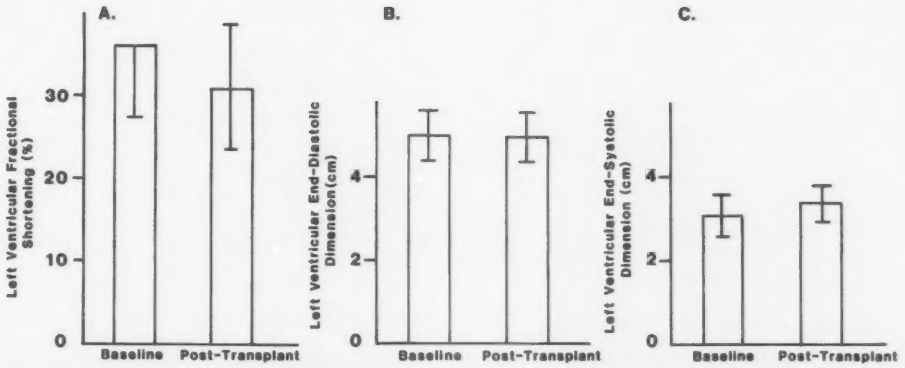

FIGURE 2.—Mean values (± SD) of % echocardiographic left ventricular fractional shortening (A) and diastolic (B) and systolic (C) minor axis dimensions at baseline and 3 wks post-transplant. No significant changes were noted in these parameters after bone marrow transplantation. Due to attrition during the study and inability to record follow-up echocardiograms in some patients, 21 patients are included in the baseline mean values, whereas 12 are included in the posttransplant values.

transplantation, no clinically significant evidence of left ventricular dysfunction was noted. However, in seven of the 17 patients (41%) who completed the study, resting left ventricular ejection fraction showed mild, subclinical deterioration between baseline and discharge. These patients could not be distinguished on the basis of prior chemotherapy or echocardiographic left ventricular diastolic dimension. In four of these patients, resting ejection fraction fell into the abnormal range. Three of these four patients had had previous anthracycline-containing chemotherapeutic regimens. In this discussion, we will place these findings in the perspective of prior observations and emphasize the clinical implications of our findings.

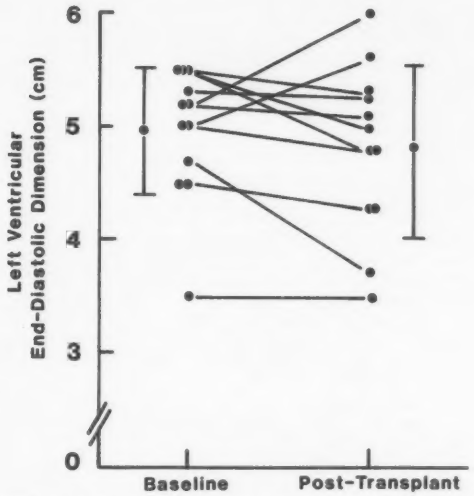

FIGURE 3.—Left ventricular minor axis end-diastolic dimensions at baseline and 3-9 wks posttransplant in the 12 patients in whom satisfactory follow-up echocardiograms could be obtained. No significant difference in mean diastolic dimension was noted.

Comparison to Prior Observations

Cardiac toxicity of cyclophosphamide manifests as cardiomyopathy secondary to a hemorrhagic myocarditis (5-8,10-12). Pathologically, interstitial edema, fibrin microthrombi, myocardial hemorrhage, and occasional acute myocardial necrosis are seen (5,6,8,10,12). Cardiac toxicity occurs with high doses of cyclophosphamide, usually > 180 mg/kg (4-6,8-13). The fact that many patients with cardiac toxicity have had concurrent chemotherapy (5-7,11,12) or previous anthracycline therapy (5-7,12) would suggest that such therapy predisposes a patient to the cardiotoxic effects of cyclophosphamide (6). No clinical evidence of cardiac toxicity was noted by Graw et al (16) in nine patients receiving 180 mg/kg of cyclophosphamide or by Mullins et al (17) in 12 patients receiving 120 mg/kg, all of whom received no accompanying chemotherapy. Furthermore, reported cases of cyclophosphamide cardiac toxicity at doses < 180 mg/kg have had previous anthracycline therapy (7) or mediastinal radiation (7). Toxicity is usually evident within 3 weeks of exposure (4-13).

The cardiac toxicity of 180 mg/kg of cyclophosphamide over 4 days was well-delineated by Gottdiener et al (6). They followed 32 patients with serial electrocardi-

TABLE 4.—Pathological findings in 4 patients

Patient No.	Pathologic findings
2	Dilated left ventricle; variable fibrosis of endocardium, myocardium, and epicardium, and focal plasma cellular infiltrate in the pericardium and endocardium
11	No cardiac abnormalities
12	No cardiac abnormalities
20	Bacterial endocarditis with splenic, right and left kidney, and left ventricular myocardial infarcts

ography and echocardiography. Of note, many of these patients had also received carmustine, cytarabine, and thioguanine, and eight patients had had previous doxorubicin therapy. These investigators noted clinical evidence of heart failure in 28%, with 19% dying of myocardial failure. Pericardial tamponade occurred in five of the six patients dying of myocardial failure. Fractional minor axis shortening on echocardiography was diminished in eight of 13 patients. No significant change in left ventricular diastolic minor axis dimension was noted. All changes were noted within 3 weeks, with no patient who survived past 3 weeks developing late reactions.

Cardiac complications of radiation may be immediate (within days to weeks) (2,18), short-term (within months to 1 or 2 years) (18,19), or late (many years) after the exposure (20–25). Acute cardiotoxicity of radiation has been described most often in patients receiving massive doses of radiation, most often related to atomic warfare or accidents involving radiation (2). In such patients toxicity manifests as an acute fibrinous pericarditis. The significance of various rhythm disturbances and electrocardiographic changes temporally associated with radiation is not certain (26). Short-term cardiotoxic effects of radiation are manifest most frequently as an acute organizing pericarditis with fibrotic thickening and pericardial effusion that occurs from several months to years after exposure (18,26,27). Late cardiac complications include chronic pericardial disorders, including constrictive pericarditis (21,25,28) and myocardial fibrosis with evidence of a restrictive cardiomyopathy (20,29,30).

Most available information about the cardiac complications of radiation has come from patients receiving "mantle" radiation for Hodgkin's disease (18,27) or radiation therapy for chest neoplasms or breast cancer. In studies of patients with Hodgkin's disease, radiation toxicity is most often pericardial and manifests within 2 years of exposure (18,31–33). Toxicity occurs in from 6% (18) to 30% (27) of patients. Late complications may be even more common than previously thought (20). Toxicity is dose-dependent (34) and cardiac shielding should reduce the incidence (32), although this has not been proven.

Little information is available regarding lower doses of radiation such as those used in preparative regimens for bone marrow transplantation. Since such regimens combine chemotherapy with lower doses of total-body irradiation, the possibility for the potentiation of cardiotoxic effects exists. There is evidence that enhanced cardiotoxicity exists with the combination of radiation and doxorubicin (35–39). Few data are available concerning the combination of cyclophosphamide and radiation, although in one reported case of cyclophosphamide toxicity at 168 mg/kg, the patient had had previous mantle radiation (dose unknown) for Hodgkin's disease. There have been few prospective studies comparing the efficacy and toxicity of different bone marrow transplantation preparative regimens (1).

Clinical Implications

Our study suggests that the combination of moderately high-dose cyclophosphamide and radiation does not appear to cause substantial, clinically important, short-term cardiac toxicity. Our data, however, were obtained over a short-term follow-up period in a relatively small number of patients. In some of these patients, subclinical deterioration in left ventricular function was found. In the treatment of the individual leukemic patient with bone marrow transplantation, the efficacy of the procedure must be balanced against the possibilities of many potential toxic effects. Although both chemotherapy and irradiation have known toxic effects on the heart, relatively little information is available concerning the cardiac sequelae of the particular combinations of treatment modalities used in bone marrow transplantation. Treatment regimens combining chemotherapy and irradiation are being used prior to allogeneic bone marrow transplantation in patients with acute and chronic leukemias. The preparative regimen used in this study has previously been shown to be relatively effective in the treatment of leukemia (14). The present data suggest that this regimen has little apparent short-term cardiac toxicity in the majority of adult patients. These findings are in contrast to the results of previous studies using higher doses of cyclophosphamide (6). Thus, we conclude that the treatment regimen described in this study may be used without major short-term effects on left ventricular function in most patients. The potential for long-term effects of the regimen on cardiac performance will remain unclear until continued surveillance of patients undergoing bone marrow transplantation is accomplished.

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Phase II Study of Carboplatin in Patients With Ovarian Carcinoma: A National Cancer Institute of Canada Clinical Trials Group Study[1,2]

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The National Cancer Institute of Canada Clinical Trials Group conducted a phase II study of carboplatin (400 mg/m^2) given as an iv bolus every 4 weeks in patients with measurable advanced ovarian cancer who had failed or relapsed following standard platinum-containing therapy. Four complete and eight partial responses were seen in 43 evaluable patients. Toxicity was primarily hematologic. Myelosuppression, particularly thrombocytopenia, was severe in one-third of patients treated at the 400-mg/m^2 starting dose. Carboplatin has antitumor activity in this clinical setting, but a lower starting dose is recommended. [Cancer Treat Rep 70:1195–1198, 1986]

The advent of cisplatin-containing combination chemotherapy has provided improvement in the response rates for patients with advanced ovarian carcinoma, but seldom cures such patients. The efficacy of secondary chemotherapy in women who fail to respond to or who relapse after cisplatin-containing primary treatment is extremely poor. For example, in a recently completed randomized trial by the Clinical Trials Group of the National Cancer Institute of Canada (NCIC), < 5% of patients exhibited objective responses to second-line therapy with melphalan with or without hexamethylmelamine (unpublished data).

As part of an ongoing effort to identify active new agents for patients with relapsed ovarian cancer, the NCIC has recently completed a phase II trial of the cisplatin analogue carboplatin. Carboplatin is a second-generation cisplatin analogue developed in an effort to increase the therapeutic ratio of the parent compound. Phase I studies of carboplatin found it caused no nephrotoxicity. However, both myelosuppression (particularly thrombocytopenia) and moderate nausea and vomiting were seen (1–3). In phase II trials in ovarian cancer, responses were observed in patients who were both previously untreated and previously treated with cisplatin (4,5). This study examines the effect of a relatively high dose of carboplatin (400 mg/m^2) given as second-line therapy in a population of ovarian cancer patients with prior cisplatin treatment.

MATERIALS AND METHODS

Between August 1984 and February 1985, 53 patients were entered onto this phase II study. Eligibility criteria included histologically proven epithelial ovarian carcinoma with measurable advanced disease, Eastern Cooperative Oncology Group performance status of 2 or better, absolute granulocyte count $\geq 2.0 \times 10^9$/L, platelet count $\geq 150 \times 10^9$/L, creatinine clearance > 1 ml/sec (60 ml/min), and bilirubin < 1.5 times normal. Patients had to have had a maximum of one prior chemotherapy regimen which must have contained cisplatin. That is, carboplatin was to be second-line chemotherapy. Patients who had bowel obstruction were considered ineligible. Entry into the study was not restricted by the nature of the response to first-line therapy or by prior radiation therapy.

Informed consent was obtained from all patients. Carboplatin was supplied by the Investigational Drug

[1] Received Feb 25, 1986; revised June 2, 1986; accepted June 23, 1986.
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Branch, National Cancer Institute, Bethesda, MD. The drug was administered at a starting dose of 400 mg/m^2, except in the instance of prior radiation, when a starting dose of 320 mg/m^2 was permitted. The reconstituted drug was diluted in 100 ml of 5% dextrose in water and injected iv over 30 minutes. Antiemetic therapy was given in all participating centers, but was not standardized for the purpose of this trial. Treatment was repeated every 4 weeks until disease progression or unacceptable toxicity was encountered.

The dose of carboplatin in subsequent cycles was reduced by 20% if the platelet count nadir fell to $< 50 \times 10^9$/L or the granulocyte count fell to $< 0.5 \times 10^9$/L. The carboplatin dose was escalated to 450 mg/m^2 if platelet and granulocyte count nadirs were $> 100 \times 10^9$/L and 1.0×10^9/L, respectively, providing that treatment day counts were normal.

While on study, patients had a medical history and physical examination repeated every 4 weeks. Appropriate radiologic studies were repeated every 4 to 8 weeks to assess response. Hematologic parameters, renal function, and blood chemistry determinations were assessed at frequent intervals.

Response criteria were as follows: *complete response*—the complete disappearance of all clinical evidence of active tumor for a minimum of 4 weeks; *partial response*—a $\geq$ 50% decrease in the sum of the products of the diameters of measurable lesions for a minimum of 4 weeks; *progressive disease*—a $\geq$ 25% increase in the size of any measurable lesion or the appearance of new lesions; and *stable disease*—disease regression of $<$ 50% or increase of $<$ 25% for a minimum duration of 8 weeks. Response duration was measured from the point in time that response criteria were first achieved until disease progression was documented.

RESULTS

Of the 53 patients entered onto the trial, only 44 were eligible (table 1). The majority of ineligible patients were more extensively pretreated than was stipulated in the protocol. When this was identified as a problem in the first weeks of the study, the protocol was clarified and no such heavily pretreated patients were entered subsequently.

The characteristics of the 44 eligible patients are described in table 1. All patients were previously treated with one cisplatin-containing regimen. The first-line regimen was not standardized, but in all but six cases consisted of cisplatin (50 mg/m^2) with additional doxorubicin (50 mg/m^2) and/or cyclophosphamide (500 mg/m^2) repeated in 3–4-week cycles. In the remainder, higher doses of cisplatin were used in combination regimens.

The response to first-line therapy is shown in table 1. Approximately one-half of the patients had had either a complete or partial response to their cisplatin-containing regimen. The median time from the end of first-line therapy to the beginning of carboplatin treatment was 3 months (range, 4 weeks to 39 months).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	53
Eligible	44
Reasons for ineligibility	
> 1 prior chemotherapy	6
Bowel obstruction	1
Performance status > 2	1
Hematologic dysfunction	1
Median age in yrs (range): 57 (33–71)	
Performance status*	
0	14
1	17
2	13
Prior radiation therapy	10
Response to first-line chemotherapy	
Complete response (histologic)	3
Complete response (clinical)	2
Partial response	21
Stable disease	6
Progressive disease	10
Not evaluable	2
Total	44
Interval (mos) between first-line regimen and carboplatin	
≤ 2	20
> 2	24
Cycles of carboplatin	
1	9
2	13
3	8
≥ 4	14

*Eastern Cooperative Oncology Group criteria.

The number of treatment cycles of carboplatin is shown in table 1. One-half of the patients received three or more cycles. The maximum number of cycles received by any patient in this trial was 11. Of those receiving less than three cycles, all but three patients went off treatment because of disease progression.

Toxicity

The toxicity of carboplatin was consistent with that observed in phase I studies, that is, primarily myelosuppression, nausea, and vomiting. The neutropenia and thrombocytopenia observed at the 400-mg/m^2 and 310–340-mg/m^2 dose levels are described in tables 2 and 3. In table 2, the hematologic toxicity encountered in 111 cycles of treatment is described. In table 3, the numbers of patients ever to experience severe hematologic toxicity are documented at the same two dosage levels. Thirty-eight patients are evaluable at the 400-mg/m^2 dose level (starting doses were reduced in six cases because of prior radiation and/or physician discretion) and 18 are evaluable at the 310–340-mg/m^2 dose level. Only three patients could be escalated to 450 mg/m^2 and that dose level is not included in the tables.

Thrombocytopenia was seen more frequently than

TABLE 2.—Hematologic toxicity by cycle

	Dose level (mg/m^2)	
	400	310–340
No. of cycles evaluable		
Total	72	39
For granulocyte nadir	61	37
For platelet nadir	57	37
Median nadir granulocyte count (range)*	1.6 (0.08–4.7)	1.8 (0.7–3.5)
Median nadir platelet count (range)*	61 (3–244)	76 (10–308)
No. of cycles (%) with—		
Granulocyte counts < 0.5*	6 (10)	0
Platelet counts < 50*	24 (42)	(27)
Platelet counts < 25*	12 (21)	(8)

*$\times 10^9$ cells/L.

neutropenia at both dose levels and its severity was dose-related. At 400 mg/m^2, 21% of cycles were accompanied by nadir platelet counts of $< 25 \times 10^9$/L. The corresponding figure at the 310–340-mg/m^2 dose level was only 8%.

It is also important to examine the toxicity by patient at each dose level (table 3). At the 400-mg/m^2 dose level, 12 of 38 patients (31%) developed nadir platelet counts of $< 25 \times 10^9$/L during their treatment. Bleeding was documented in three patients on study and may have contributed to the death of one patient.

Neutropenia was also dose-related, but was not as frequently severe as thrombocytopenia. At the 400-mg/m^2 dose level, neutropenia of $< 0.5 \times 10^9$ cells/L was seen in 10% of cycles (table 2). None of the carboplatin cycles given at a dose of 310–340 mg/m^2 were accompanied by this degree of neutropenia. One patient developed an *Escherichia coli* septicemia while neutropenic, but recovered with appropriate antimicrobial therapy.

The nadir granulocyte count tended to occur slightly later in the cycle than the nadir platelet count (Day 20 vs Day 15 median). Approximately 20% of all treatment cycles had to be delayed to allow recovery of cytopenias. Thirty percent of cycles at 400 mg/m^2 and 13% of cycles at 310–340 mg/m^2 resulted in myelosuppression requiring dose reduction in subsequent cycles.

Anemia was also encountered frequently on the study. Approximately 80% of patients required transfusion support by the end of the third cycle of carboplatin. In three patients, bleeding contributed to the severity of the anemia, but there was no obvious blood loss or hemolysis to account for the fall in hemoglobin in the remainder.

Mild to moderate nausea and vomiting occurred in two-thirds of the patients on this study. A few patients (16%) were recorded as having severe nausea and vomiting. A total of five patients went off study for reasons of toxicity alone: three because of nausea and vomiting, one because of diarrhea and headache, and one because of generalized weakness. All toxic effects were attributed to carboplatin. No neuropathy or renal toxicity was seen.

Response

Forty-three patients were evaluable. The only inevaluable patient died of pulmonary embolism on Day 10 of her first treatment cycle.

Twelve of the 43 patients had an objective response to treatment (28%). Four patients had complete clinical responses and eight had partial responses. In the group of 12 responders, eight had had a complete or partial response to their first-line cisplatin-containing combination therapy and two had had progressive disease. The median time to response was two cycles, with up to six cycles required before response criteria were met. Nine patients had stable disease and 22 had progressive disease.

In general, those patients who had complete responses had low-bulk disease when compared to the remainder of the study population, although this was not quantified accurately by pretreatment laparotomy. Also of interest is that, in the group of 20 patients with a short (≤ 2 months) interval between prior cisplatin therapy and carboplatin treatment, only two (partial) responses were seen. This can be contrasted with ten responses seen in 23 patients with a longer interval (> 2 months) between prior cisplatin treatment and initiation of carboplatin therapy.

Response is ongoing in four of the 12 patients. The median response duration is 4 months (range, 2–10+).

DISCUSSION

In this phase II study, carboplatin, given at a starting dose of 400 mg/m^2 every 4 weeks, produced a 28% response rate in patients with measurable ovarian carcinoma who had received one prior cisplatin-containing regimen.

This result is similar to that reported by Evans et al (5), who observed seven responses in 28 patients, all of whom had received prior therapy with cisplatin. Four of the responding patients in that study and two of the 12 in the current trial had failed to respond to cisplatin-containing therapy. While it is tempting to claim that this may be evidence that the two drugs are non-cross-resistant, it must also be emphasized that, at least in the current study, cisplatin doses were modest (50 mg/m^2

TABLE 3.—Hematologic toxicity by patient

	Dose level (mg/m^2)	
	400	310–340
No. of evaluable patients	38	18
No. of patients (%) to ever experience—		
Granulocyte counts $< 0.5 \times 10^9$ cells/L	6 (16)	0
Platelet counts $< 50 \times 10^9$ cells/L	22 (58)	10 (55)
Platelet counts $< 25 \times 10^9$ cells/L	12 (31)	3 (17)

per course in the majority of cases) and it is possible that some patients responsive to carboplatin may have demonstrated a tumor response to an escalated dose of cisplatin (6,7). The toxicity of carboplatin was primarily hematologic and gastrointestinal. Severe thrombocytopenia (platelet count $< 25 \times 10^9$/L) was documented in 12 of 38 patients and severe neutropenia ($< 0.5 \times 10^9$ cells/L) was documented in six of 38 patients treated at the 400-mg/m^2 dose level. No particular risk factor, such as previous radiation therapy, seemed to identify those patients at increased risk of myelosuppression, but the small numbers of patients in this study did not permit an extensive analysis of potential risk factors. We feel that the starting dose of carboplatin of 400 mg/m^2 per course was too high in this cisplatin-pretreated population and would therefore recommend that a lower starting dose, such as 320 mg/m^2, be used, with escalation as appropriate based on nadir blood cell counts.

In summary, carboplatin has definite antitumor activity when given as a single iv bolus every 4 weeks in patients with ovarian cancer who have received prior cisplatin therapy. Myelosuppression is considerable at 400 mg/m^2, and lower doses (320 mg/m^2) should be employed in this clinical setting in future studies. Even lower doses may be necessary when carboplatin is used as second-line therapy in less well selected groups of patients.

The final place of carboplatin in the therapeutic armamentarium for ovarian cancer will depend upon the results of several randomized comparative studies with cisplatin now ongoing in Canada and elsewhere.

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Randomized Comparison of Tamoxifen Versus Diethylstilbestrol in Estrogen Receptor-Positive or -Unknown Metastatic Breast Cancer: A Southeastern Cancer Study Group Trial[1,2]

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In a randomized study 115 postmenopausal women with advanced breast cancer who were estrogen receptor-unknown or -positive were treated initially with tamoxifen or diethylstilbestrol (DES). Their pretreatment characteristics showed no significant difference. The frequency of response was identical with tamoxifen and DES, showing a complete response rate of 2% versus 2% and a partial response rate of 4% versus 8%, respectively; stable disease was present in 78% versus 73% of the patients, respectively. The median time to disease progression (5 vs 6 months) and median survival depending on initial hormone therapy (34 vs 35 months) were identical for tamoxifen and DES, respectively. Gastrointestinal toxicity was more frequent and more severe with DES than tamoxifen. Responses were seen with withdrawal of each agent and on crossover to the alternative agent. Our conclusions are that: (*a*) DES and tamoxifen are equally effective in treating metastatic breast cancer in the postmenopausal patient who is estrogen receptor-positive or -unknown; (*b*) withdrawal and crossover responses are seen with both agents; (*c*) side effects are minimal but more frequent with DES; and (*d*) on the basis of cost-effectiveness DES is the preferable agent. [Cancer Treat Rep 70:1199–1203, 1986]

Hormonal therapy for women with metastatic breast cancer is a standard approach and in the postmenopausal patient this has traditionally begun with the addition of estrogens. In 1960, the AMA Therapeutics Trial Committee published objective response rates of 30%–40% depending on the site of predominant disease using diethylstilbestrol (DES) or ethinyl estradiol (EE) (1). In 1970, Stoll and Ackland (2) confirmed this in patients > 70 years old with a 49% tumor regression rate. However, the development and marketing of the antiestrogen tamoxifen has in large part replaced the use of exogenous estrogens. The initial studies with tamoxifen showed it to be effective and to have few serious side effects (3–5). These early studies have been confirmed by two randomized studies comparing tamoxifen versus DES or EE (6,7).

The tamoxifen versus DES study of Ingle et al (6) suggested an increased tumor regression rate for DES but the difference was not statistically significant. They argued that because there was significantly less serious toxicity (defined as severe nausea or emesis, moderate to severe edema, congestive heart failure, or phlebitis), tamoxifen should be the drug of choice. This study did not prospectively evaluate the presence of a hormone-withdrawal response, a crossover response, or the influence of estrogen receptor (ER) status. The study of Beex et al (7) comparing tamoxifen versus EE plus chlorothiazide showed no therapeutic differences in response or duration of response. They found the best response in ER-positive patients with limited disease. However, withdrawal responses were seen only with EE. They concluded that the drugs were equally effective but that tamoxifen's side effects "appeared" less serious.

The present randomized clinical trial was undertaken to compare DES versus tamoxifen in postmenopausal women to evaluate the questions of primary response, crossover response, withdrawal response, ER status in relation to response, and comparative toxic effects.

METHODS

One hundred fifteen consenting women with biopsy-proven metastatic breast cancer were entered in the

[1] Received June 11, 1985; revised June 23, 1986; accepted July 1, 1986.
[2] Supported in part by Public Health Service grants CA-03227, CA-20255, CA-03876, CA-23909, CA-03177, CA-15584, CA- 07961, CA-13237, CA-12223, CA-28143, CA-05641, CA-28138, CA-28171, CA-30247, CA-03013, CA-28169, CA-19657, and CA-24456 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[6] We thank Drs. George Omura and Charles Vogel for their helpful comments and review of the manuscript.
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study from February 1979 to September 1982 after appropriate institutional review board approval. Initial requirements were that only patients ≥ 70 years of age were eligible, but because of slow patient accrual, this was reduced to women 2 years postmenopausal or > 50 years old if surgical oophorectomy had been performed. Women with ER-positive or -unknown tumors were accepted, but ER-negative patients were excluded. No previous estrogens or antiestrogens could have been used. Patients who had received adrenalectomy, androgens, or progestational agents were eligible provided that at least 4 weeks had elapsed since prior endocrine treatment and objective evidence of disease progression was present. The patient could have had previous chemotherapy as long as acute toxic effects had subsided and progression of disease was evident. Bidimensional (Class I) or unidimensional (Class II) measurable disease was required for entry as defined in the guidelines from the breast cancer treatment program (8). Response criteria were as follows: *complete response*—the complete disappearance of all known disease for at least 4 weeks; *partial response*—a ≥ 50% decrease in measurable disease for at least 4 weeks; and *stable disease*—a < 50% decrease or < 25% increase in measurable disease over 4 weeks. Objective response for bony metastases was as defined in the guidelines. Pretreatment studies included physical examinations, routine cbc, chemistry profile, chest x-ray, and bone scan. A liver scan was required if liver function tests were abnormal.

An ER assay > 3 fmols/mg of cytosol protein was considered positive. Progesterone receptors were recorded if available. ERs were determined by different methods in the various participating institutions.

Patients were stratified by dominant disease site (soft tissue, osseous, or visceral), prior endocrine therapy, prior chemotherapy, and ER status. They were then randomized to either DES at a dose of 5 mg orally three times a day or tamoxifen at a dose of 10 mg orally twice a day.

Study patients were to be evaluated at 4 and 8 weeks and at every 12 weeks thereafter until death. Therapy was continued if there was stabilization of disease or disease regression. DES was stopped if thrombophlebitis or pulmonary embolus occurred or if nausea and vomiting persisted despite DES dose reduction. Tamoxifen was stopped for severe nausea or vomiting not responding to dose reduction or for the development of severe thrombocytopenia.

Once a patient showed disease progression on one endocrine therapy, that agent was stopped for 4 weeks. If the disease stabilized or any withdrawal response was seen, the patient was kept off therapy. If at ≥ 4 weeks after stopping one agent there was evidence of disease progression, the alternate hormone was to be started. The response to this second hormonal agent was evaluated 28 days later, and treatment was continued if there was a response or stabilization of disease.

Survival and duration of remission curves were calculated with the Kaplan and Meier techniques and compared for significance by the Gehan method (9,10). Other comparisons were made using chi-square statistics.

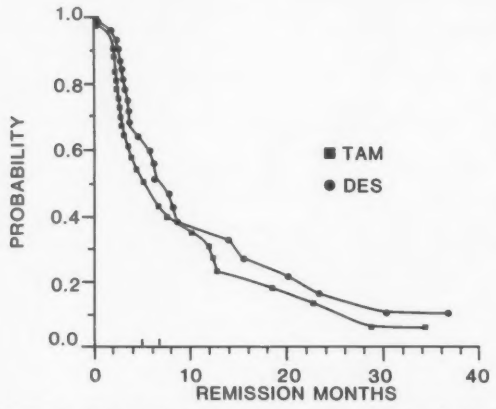

FIGURE 1.—Time to disease progression of all patients evaluable for response who were stable or better with induction hormonal therapy. There is no statistically significant difference between DES and tamoxifen (TAM).

	Total	Relapsed	Censored	Median
TAM	46	27	19	5 mos
DES	44	22	22	6 mos

RESULTS

There were 115 women entered in the study, of whom 102 were evaluable for response and 104 for toxicity. Two patients were not eligible for study, three had major protocol violations, six had inadequate data reported, and two had inadequate response data. At 4 weeks from the start of therapy, complete response rate, partial response rate, and stable disease were almost identical between DES and tamoxifen. Ninety-four patients were continued on therapy, of whom five were not eligible. One patient was ineligible for induction and four progressed during induction. Figure 1 shows time to progression of disease for all patients who achieved at least a stable response at the end of 4 weeks of therapy. There was no significant difference between DES and tamoxifen. The median duration of a stable or better response for tamoxifen was 5 months and for DES was 6 months, which is not statistically different. The frequency of response between the two agents was identical for tamoxifen and DES, showing a complete response rate of 2% versus 2% and a partial response rate of 4% versus 8%, respectively. Stable disease was present in 78% of the tamoxifen patients versus 73% of the DES patients. The response which was achieved at any point during protocol therapy (initial hormone response, withdrawal response, crossover response) was no different between tamoxifen and DES: complete response rate of 6% versus 8% and partial response rate of 11% versus 17%, respectively. Stable disease was present in 67% of the tamoxifen patients versus 69% of the DES patients and disease progression was present in 17% versus 3%, respectively.

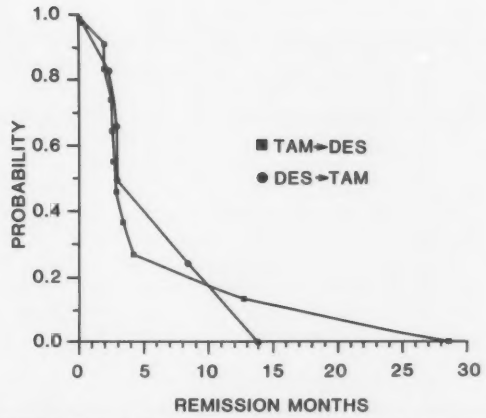

FIGURE 2.—Time to disease progression of all patients after crossover to the alternate therapy. There is no statistically significant difference between DES and tamoxifen (TAM).

	Total	Relapsed	Censored	Median
TAM → DES	13	10	3	2.7 mos
DES → TAM	11	5	6	2.8 mos

Due to poor protocol compliance only 17 patients could be evaluated for a withdrawal response. These patients met the criteria of being off hormone therapy for 4 weeks and being evaluated for disease response at that point and thereafter. In this small group of patients it is impossible to draw any definite conclusions except that one partial response was seen after DES withdrawal and four patients had stabilization of disease. One patient had stabilization of disease after tamoxifen withdrawal.

Thirty-five patients were evaluated in the crossover arm with no complete response seen and one partial response with crossover from tamoxifen to DES. There was equal percent of stable disease (67% tamoxifen to DES versus 65% DES to tamoxifen). There was no significant difference in progression rates with the crossover therapies (fig 2). The median duration of response was 50 days, with a range of 19–297 days.

The overall survival for all eligible randomized patients in the study is shown in figure 3 and shows no difference between tamoxifen and DES.

There were no significant differences found in pretreatment characteristics between the two groups with all P values > 0.10 for comparisons as shown in table 1. Patient characteristics of ER status, extensiveness of disease, age, length of time since menopause, disease-free interval, dominant location of metastatic disease, performance status, and previous treatment did not correlate to response rates or to stable disease. There is a suggestion that the presence of ER is associated with DES response (100%) more frequently than a tamoxifen response (85%), but the difference is not statistically significant ($P = 0.80$). Our data are inadequate to determine if there is an ER level which is predictive of hormone responsiveness.

Toxic effects associated with the hormone therapy are shown in table 2. Gastrointestinal toxicity is more frequent with DES (12 patients vs three) and the severity is greater, with two moderate and three severe versus only mild with tamoxifen. The three patients receiving DES who developed congestive heart failure had many other medical problems in their clinical course and it did not appear that DES was related to their congestive heart failure. No thrombocytopenia was encountered with tamoxifen or phlebitis with DES. One patient had a stroke while receiving DES, another had right arm paresthesia while receiving tamoxifen, and another had psychotic symptoms with both agents.

The study was designed to detect at least a 20% increase in response rate based on a 10% one-sided test of significance, an underlying binomial distribution, and a power of 80%. This required 60 patients per arm. The actual observed response rate fell below expected levels for both arms and the study was discontinued with approximately 50 patients on each arm.

DISCUSSION

The present study confirms the data from the previous two randomized studies that tamoxifen is an effective agent in the treatment of breast cancer (6,7). The frequency of response (complete and partial) and stable disease in the study by Ingle et al (6) was 84% for DES and 78% for tamoxifen, which is comparable to our results of 83% for DES and 82% for tamoxifen. The median survival was 31 months in the study of Beex et al (7) and 34.5 months in the present study. In all studies there was a slight but statistically insignificant survival advantage for DES or EE over tamoxifen.

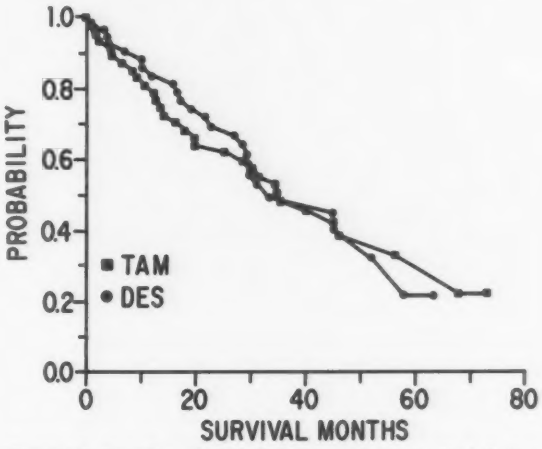

FIGURE 3.—Overall survival of all eligible randomized patients in the study. TAM = tamoxifen.

	Total	Relapsed	Censored	Median
TAM	54	25	29	34 mos
DES	48	20	28	35 mos

TABLE 1.—Pretreatment characteristics*

Characteristic	DES patients	Tamoxifen patients
Total	50	54
Disease-free interval (DFI)†		
Patients without DFI	13	17
Patients with DFI	37	37
Median duration (mos)	38.7	49.9
Range (mos)	1.5-294	2-204
Age (yrs)		
Median	71.2	69.6
Range	45-86	41-95
Menopausal period (yrs)‡		
Median	21	20
Range	2-50	2-50
Dominant localization of metastases		
Soft tissue	13	17
Bone	25	24
Viscera	12	13
Extent of disease§		
1 site	11	6
2-3 sites	26	30
> 3 sites	13	14
ER activity		
Positive	30	34
Unknown	20	20
Previous treatment		
Hormonal and chemotherapy	0	2
Hormones alone	0	0
Chemotherapy alone	5	7
No chemotherapy or hormones	45	45
Adrenalectomy/hypophysectomy	2	0
Interval between diagnosis of recurrent disease and start of treatment (mos)¶		
Median	1.4	2.4
Range	0-26.9	0-13.8
Performance status at start of treatment		
70-100	42	47
40-69	6	7
< 40	2	0

*Unless otherwise specified, values = No. of patients.

†Time from initial diagnosis to time of documented metastatic disease.

‡Time from cessation of menses to onstudy. 6 patients in each group had incomplete data.

§Extent of disease at time of study as an attempt to estimate total-body tumor burden. 4 patients in tamoxifen group had incomplete data.

¶10 patients in DES group and 14 patients in tamoxifen group had incomplete data.

Tamoxifen is no better than DES in control of metastatic breast cancer. Although toxicity with DES is more frequent, it usually occurs in the first 4 weeks of therapy and is mild in severity. The cost differential between the two agents is large, with the daily cost of tamoxifen seven times that of DES.

In this age group the usual predictors of response were not helpful. The ER-positive patients in this study did not show an improved response rate over those who were ER-unknown.

Neither agent in this study resulted in as many partial or complete responses as had been previously found. Ingle et al (6) reported an 11% complete response rate and a 30% partial response rate for DES and a 9% complete response rate and a 25% partial response rate for tamoxifen. This study found only a 2% complete response rate and an 8% partial response rate for DES and a 2% complete response rate and a 4% partial response rate for tamoxifen. The reasons for these differences are not entirely clear. However, given that the median time to disease progression was 5–6 months in the study of Ingle et al and 6–7 months in this study these differences in frequency of response may reflect a difference between the studies in the definition of response. In this study nonmeasurable bone disease was allowed, with a response being accepted only with evidence of healing of a lytic bone lesion. This was a rare event and probably resulted in a lower frequency of response since 50% of our patients had bone as the dominant site of disease at initial presentation.

Protocol compliance was poor regarding the evaluation of withdrawal response so that no definitive conclusion can be made. However, withdrawal responses and stabilization of disease were seen with DES and tamoxifen; therefore, a period of observation prior to starting a new therapy after failure of these agents is warranted.

The crossover responses seen with both agents were of similar frequency and of moderate duration, supporting this approach in hormone-responsive patients. The crossover responses were all maintenance of stable disease.

Side effects (nausea, emesis, leg edema, and breast tenderness) from DES were more frequent than with tamoxifen (table 2). These were, in general, mild and not life-threatening. No thrombocytopenia, phlebitis, or stress incontinence was recorded in this study. Minor side effects must be weighed against the sevenfold increase in drug cost with no gain in frequency or duration of disease control in choosing tamoxifen rather than DES. This study confirms the equivalency of DES and

TABLE 2.—Toxic effects*

	DES patients		Tamoxifen patients	
	Primary	Crossover	Primary	Crossover
Nausea/emesis	10	1	2	1
Diarrhea	1			
Edema	5		1	
Congestive heart failure	3			
Hot flashes	1			
Vaginal spotting		3	2	
Hypercalcemia		2	2	
Withdrawal vaginal bleeding	1			
Breast tenderness	4			
Liver	2		1	
Skin and hair			1	
Weight loss		1	2	
CNS	1	1	1	

*Values = No. of patients.

tamoxifen and suggests that a crossover trial of these agents should be considered in patient management. On the basis of cost-effectiveness, it is our opinion that DES is the agent of choice as initial therapy in the ER-positive or -unknown postmenopausal woman with metastatic breast cancer.

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Effects of Intraventricular Methotrexate on Cerebrospinal Fluid Monoamine Metabolites in Rhesus Monkeys[1,2]

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Previous work suggested that methotrexate (MTX) may cause encephalopathy by inhibiting biosynthetic pathways for dopamine and serotonin in the brain. We examined this issue by measuring the neurotransmitter metabolites homovanillic acid and 5-hydroxyindoleacetic acid in the lumbar CSF of rhesus monkeys receiving continuous intracerebroventricular infusions of MTX or dichloromethotrexate. Infusion of the lowest dose (0.05 mg/day) produced a large (300%) rise in homovanillic acid levels and a modest elevation in 5-hydroxyindoleacetic acid. During higher dose infusion, which was associated with clinical encephalopathy, the biogenic amine metabolites fell from their previous elevated levels. In one encephalopathic monkey, an injection of 1 mg of leucovorin produced a marked elevation in CSF monoamine metabolites within 1 hour and rapid clinical recovery. In contrast, leucovorin produced no change in monoamine metabolites in control animals. The data suggest that MTX may block egress of monoamine metabolites from CSF at the lower doses and suppress neurotransmitter turnover at toxic doses which cause encephalopathy. Serial measurements of CSF monoamine metabolites deserve further investigation as biochemical markers for toxic effects of MTX on neuronal metabolism in the CNS. [Cancer Treat Rep 70:1205–1209, 1986]

Patients treated with high doses of methotrexate (MTX) given intravenously (iv), intrathecally, or intraventricularly may experience meningismus, fever, somnolence, confusion, and memory loss (1–3). These acute or subacute neurotoxic effects usually remit if the intensity of therapy is reduced, although a more chronic, severe leukoencephalopathy may evolve over a period of months (4–7). Little is known about the pathogenesis of MTX toxicity in the brain, especially about direct effects on neurons. However, axonal swelling is one of the earliest neuropathological signs seen during development of leukoencephalopathy, and some toxic effects have been ascribed to alterations in biogenic amine (eg, dopamine and serotonin) metabolism (8,9).

Dopamine, other catecholamines, and serotonin are synthesized in neuronal terminals by amino acid hydroxylation, which is dependent on adequate levels of reduced tetrahydrobiopterin cofactor (BH4) (10–12). Normally, the concentration of cofactor is thought to be rate limiting. MTX is inhibitory for dihydropteridine reductase (DPR), which synthesizes most BH4, and it has been proposed that neurochemical changes in patients treated with high-dose MTX might resemble those seen in children with an inherited deficiency of this enzyme (8,13–16). These children have deficiencies of catecholamine and serotonin neurotransmitters in the brain and their metabolites in the CSF, along with an atypical form of phenylketonuria. They have neurological abnormalities which are partially reversible with neurotransmitter precursor therapy (15). Patients treated with MTX for psoriasis or childhood leukemia also have been reported to have alterations in serum and urinary changes in biopterin levels (8,10,16,17). Also, MTX causes defective BH4 regeneration in liver, consistent with inhibition of DPR (12). Therefore, there is some basis for the hypothesis that MTX might suppress synthesis of serotonin and dopamine in the brain (8,9,18).

We studied this question by measuring lumbar CSF

[1] Received Nov 7, 1985; revised June 3, 1986; accepted July 1, 1986.

[2] Supported in part by the Children's Leukemia Foundation of Michigan; Public Health Service grant NS-00603 (*M. V. Johnston*) from the National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Department of Health and Human Services; and a fellowship from the Medical Research Council of Canada (*F. S. Silverstein*).

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concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), metabolites of dopamine and serotonin, in rhesus monkeys being treated with continuous infusion of MTX into one lateral ventricle of the brain. The data have been reported previously in abstract form (19).

METHODS

A total of 11 rhesus monkeys were studied. Three were unoperated controls, and two had implanted infusion pumps and catheters but had not received any drug intraventricularly. Four were studied at multiple intervals during infusions of MTX (monkeys 537 and 744) or a more lipid-soluble analogue, dichloromethotrexate (DCMTX) (monkeys 558 and 567). Much more limited observations were made on two other monkeys that received DCMTX (monkey 564) and MTX (monkey 164A). An intraventricular silastic catheter connected to a Rickham reservoir was attached to a sc Infusaid model-100 pump (Metal Bellows Corp, Sharon, MA) implanted as described previously (20). Adult monkeys (4–7 kg) were anesthetized with im ketamine for the initial pump placement and for subsequent pump refilling and lumbar punctures. Intraventricular placement was verified by CSF withdrawal and nuclear cisternal scan using ^{169}Yb-diethyltriamine pentaacetic acid.

The pump was originally filled with Elliott's B artificial CSF solution, and when complete healing occurred, the pump reservoir was aspirated percutaneously and refilled with MTX or DCMTX dissolved in Elliott's B. Doses of 0.05, 0.5, and 5 mg/day were infused in volumes of 1–2 ml/24 hours, < 5% of the CSF produced in the monkey in that time. Each 5–7-day infusion period at a given dose was either followed immediately by the next higher dose or by a 5–7-day rest period during which CSF alone was infused. Monkeys were monitored daily for signs of neurotoxicity, and periodic CSF samples were obtained by lumbar puncture. Lumbar CSF specimens from control animals were collected under ketamine anesthesia in the same fashion as the drug-treated animals.

The CSF samples were frozen at −20°C until the time of assays. MTX and DCMTX concentrations were determined enzymatically by measuring inhibition of dihydrofolate reductase (DHFR) (21). MTX levels were not available from some of the animals because of technical problems with the assay or insufficient sample size. CSF HVA and 5-HIAA were measured using high-performance liquid chromatography with electrochemical detection (22).

RESULTS

The values for HVA and 5-HIAA in lumbar CSF from control monkeys not treated with chemotherapy were similar to each other (table 1) and close to previously reported values (23). Infusion of MTX or DCMTX at the lowest nominal rate (0.05 mg/day) for 5 days raised the lumbar CSF level of HVA more than three-fold compared with CSF from untreated monkeys (table 1). CSF MTX concentrations during the low-dose infusions were in the range of $5–8 \times 10^{-9}$ M in two of the animals (No. 537, fig 1; No. 164A, data not shown). This effect is shown in figure 2A and 2B in two animals treated with DCMTX and in figure 2C for a monkey treated with MTX. Infusion of 0.05 mg/day raised the HVA concentration by 308% and 566% in the DCMTX-treated animals (fig 2A and 2B). However, 5-HIAA did not rise to a significant degree.

TABLE 1.—Monoamine metabolites in rhesus monkey lumbar CSF after low-dose MTX or DCMTX infusion*

Monoamine metabolite	Controls ($n = 5$)	After 5-day infusion ($n = 4$)	% Change
HVA	11.6 ± 5	47 ± 6†	+310
5-HIAA	10.4 ± 2	17 ± 4	+63

*The 5 control monkeys received no prior intraventricular chemotherapy; two had pumps in place and three were unoperated. The treated group received 5 days of either MTX (monkeys 537 and 744) or DCMTX (monkeys 558 and 567) through the constant infusion pump at a rate of 0.05 mg/day as described in the Methods section. Values are mean ± SEM. Complete data from MTX-treated monkeys 567 and 558 are shown in fig 2A and 2B and data from the DCMTX-treated monkeys 558 and 567 are shown in figs 1 and 2C. Data from two additional monkeys are described in the text: No. 564 received DCMTX and No. 164A received MTX. However, data are not available from these monkeys during the 0.05-mg/day infusion and therefore they are not included in the table or figures.

†$P < 0.001$, Student's t-test.

Although the low-dose infusion of either drug raised the HVA concentration markedly, continuation of the infusion at a tenfold higher dose (0.5 mg/day) failed to cause further elevation in three of the four animals and their levels fell moderately (figs 1, 2A, and 2B). In the fourth animal, HVA did continue to climb (fig 2C). The CSF MTX concentrations during the infusion of 0.5 mg/day were in the range of $0.6–2 \times 10^{-6}$ M in two animals (No. 537, fig 1; No. 744, fig 2C). Changes in 5-HIAA were not linked to HVA. Although 5-HIAA levels rose during the course of chemotherapy, they lagged behind HVA in three of the animals (figs 1, 2A, and 2B) or fell as HVA increased (fig 2C).

We obtained adequate CSF for analysis in two monkeys during infusion of MTX at the highest dose of 5 mg/day (figs 1 and 2C). The monoamine concentrations did not achieve the levels reached during the lower dose infusions and, in fact, the concentration of HVA clearly declined in one animal (fig 1). Concentrations of 5-HIAA were also considerably lower then those attained at lower doses of MTX.

Severe encephalopathy did not occur in the four monkeys studied in detail (figs 1 and 2), though one of them (fig 1) was lethargic and anorexic at the end of the 5-mg/day infusion period. An additional monkey (No. 564) did develop signs compatible with acute MTX encephalopathy, including anorexia, ataxia, and severe lethargy. Data from this animal are not presented in a figure

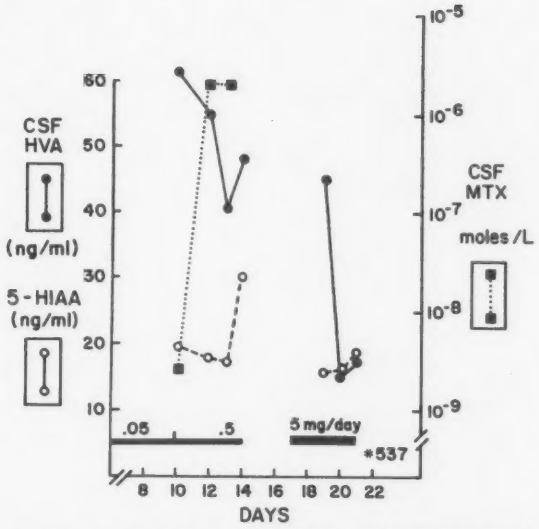

FIGURE 1.—Relationship between CSF monoamine metabolites and intraventricular MTX infusion in monkey 537. Five days before the start of the 0.5-mg/day infusion, the monkey had received a 5-day infusion of MTX at a dose of 0.05 mg/day, accounting for the nanomolar levels of MTX at the beginning of the 0.5-mg/day infusion.

because relatively few CSF samples were analyzed. However, this case was quite interesting from a therapeutic standpoint. The animal had received progressively increasing doses of DCMTX (0.05–5 mg/day) over the previous 14 days without intervening rest periods and seemed gravely ill. Levels of HVA and 5-HIAA during this period were relatively low, 6.7 and 8.8 ng/ml, respectively, but rose markedly to an HVA level of 208 ng/ml and a 5-HIAA level of 58 ng/ml within an hour after treatment with im leucovorin (1 mg). This treatment led to rapid clinical recovery, and five CSF specimens collected over a period from 12 to 25 days later contained HVA concentrations ranging from 11 to 18 ng/ml and 5-HIAA concentrations ranging from 13 to 18 ng/ml. This observation suggested that leucovorin reversed the effects of DCMTX and increased monoamine turnover. We also explored an alternative possibility, that the vitamin itself raised monoamine metabolites in the absence of the antimetabolite drug. CSF monoamine metabolites were measured in two control monkeys 1 hour after leucovorin treatment, but there was no significant change (data not shown).

DISCUSSION

Although our initial hypothesis predicted that MTX might reduce the concentration of neurotransmitter metabolites in CSF, we found instead that the lowest dose infusion of 0.05 mg/day raised HVA levels markedly. This effect occurred at a CSF drug concentration in the nanomolar range during infusion of MTX or its chlorinated, lipid-soluble analogue. The stimulatory effect was much greater for HVA than for 5-HIAA. These results are probably relevant to effects of intrathecal chemotherapy in humans. Previous work showed that the CSF MTX levels observed in the monkeys are also reached during intrathecal MTX for leukemia (24). We found in another study that CSF HVA and 5-HIAA rose significantly in children with acute lymphoblastic leukemia (ALL) treated with intrathecal chemotherapy during induction and intensification (25). We also measured extremely high concentrations of HVA and 5-HIAA in an infant with ALL treated with intrathecal MTX (F. S. Silverstein and M. Johnston, unpublished results).

Infusion of the higher doses of MTX or DCMTX produced less consistent responses than the lowest dose. In three monkeys (figs 1, 2A, and 2B), the HVA level fell, while in one (monkey 744, fig 2C) it continued to rise. Nevertheless, during infusion of the highest dose given to two monkeys, the metabolite levels returned towards normal or low levels. Therefore, these limited data suggest that the dose-response relationship between MTX infusion and monoamine metabolites may be complicated and parabolic. These studies leave unanswered whether continuation of the low-dose infusion for a longer period would have stimulated a sustained elevation in the metabolites.

The mechanism by which MTX elevates CSF HVA is unclear. CSF concentrations of HVA and 5-HIAA in lumbar CSF are dependent on several processes: neuronal turnover of the parent neurotransmitters; their rate of catabolism by monoamine oxidase and catechol-*O*-methyl transferase; transit through the CSF pathways; and removal across the blood-brain barrier by an organic acid pump (26). There is little evidence to suggest that MTX stimulates neurotransmitter turnover, although studies in neuroblastoma cells found that the antimetabolite raised the levels of BH4 (27). However, in pheochromocytoma cells, MTX in doses sufficient to inhibit DPR had no effect on conversion of tyrosine to dihydroxyphenylacetic acid (28). Also, direct brain tissue measurements of HVA and 5-HIAA after intraventricular MTX sufficient to cause leukoencephalopathy in rats did not stimulate a significant elevation of metabolites (29). Therefore, these studies suggest that the drug may elevate metabolites by blocking their egress from CSF. Since MTX is eliminated in part by an active transport system blockable with probenecid (30–32), it seems possible that the low doses of MTX, producing nanomolar concentrations in CSF, might compete with the metabolites at active transport sites. MTX might also have a direct toxic action on the acid pump.

It has been proposed that high doses of MTX placed directly into the lateral ventricles might suppress monoamine metabolite levels by inhibiting production of BH4. As noted above, experimental evidence related to this proposal is conflicting. MTX in doses sufficient to inhibit DHFR completely did not inhibit DPR activity in rat brain and in neuroblastoma cells (27,33). Other recent evidence also suggests that DHFR plays a minor role in BH4 synthesis (8,14). However, in cultured rat pineal

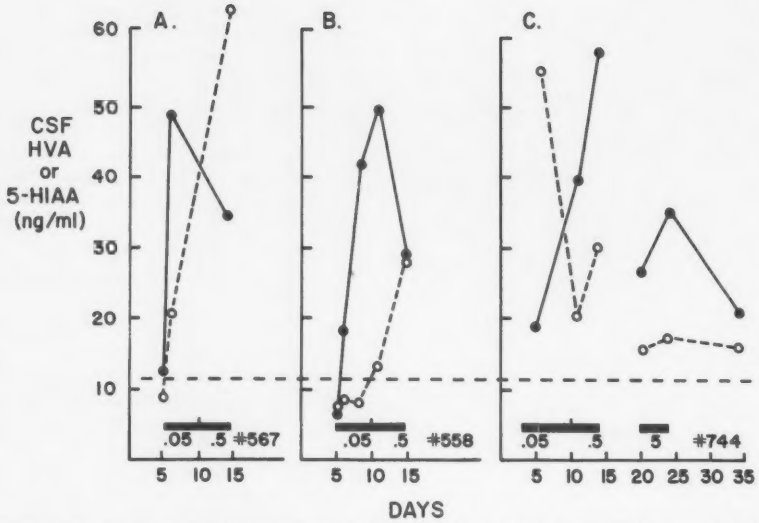

FIGURE 2.—Lumbar CSF levels of the monoamine metabolites HVA (solid circles) and 5-HIAA (open circles) during intraventricular infusion of DCMTX (panels A and B) or MTX (panel C). The numbers below the bars indicate the amount of drug calculated to be delivered per day. CSF MTX concentrations were in the nanomolar range during the 0.05-mg/day infusion and the micromolar range for the 0.5-mg/day rate (described in the Results section). The broken horizontal line shows the concentration of HVA in control animals: 11.6 ± 5 ng/ml (mean ± SEM; table 1). The normal mean concentration of 5-HIAA is close to this value (10.4 ± 2).

glands, a concentration of MTX in the same range reached in the monkeys (10 μM) for 24 hours reduced BH4 levels by 30%, presumably by inhibition of DPR (14). The biopterin cofactor in the brain is highly concentrated in catecholamine nerve terminals in the caudate and putamen, which are relatively close to the ventricular surface (34). These regions are a major source of HVA in CSF, while they contribute approximately 50% to CSF 5-HIAA (23). The other half of 5-HIAA emanates from serotonin nerve endings in the spinal cord. Therefore, suppression of monoamine turnover, dopamine more than serotonin, could possibly occur if enough MTX penetrated into the periventricular brain. Our data might be interpreted to support, though not confirm, this hypothesis. The higher dose infusions which produced micromolar MTX concentrations over several days were associated with a return of monoamine concentrations to lower levels.

The data also suggest that MTX suppressed monoamine metabolism in the monkey with severe encephalopathy (No. 564, described in the Results section). During the period of severe illness, CSF monoamine levels were relatively low and a small dose of leucovorin rapidly corrected the clinical condition and elevated the metabolites markedly. The mechanism for this dramatic reversal is not clear. Since leucovorin did not raise CSF monoamine levels in control monkeys, we propose that folate therapy may have reversed inhibition of monoamine synthesis.

It is noteworthy that in children with leukemia we also found low metabolite levels in several patients with signs of encephalopathy (24). The mechanism by which antifolate treatment inhibited monoamine metabolism and caused encephalopathy is unknown but may be entirely independent of effects on biopterin metabolism. Intrathecal cytarabine may also cause encephalopathy and suppress monoamine metabolism. A common action of this drug and MTX is to suppress protein synthesis (24).

These data from monkeys and other reports from patients provide support for a link between neurotransmitter changes in the CSF and behavioral abnormalities in acute or subacute MTX encephalopathy. Our studies did not address the relationship between these neurotransmitter changes and chronic leukoencephalopathy. Nevertheless, serial measurements of CSF metabolites during chemotherapy deserve further investigation as biochemical markers for effects of chemotherapy on neuronal metabolism in the brain.

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BRIEF REPORTS

Dose-Response Relationship to Dacarbazine Demonstrated in a Patient With Malignant Melanoma[1]

Karen L. Hoelzer, Bruce R. Harrison, Dan W. Luedke,* and Banti Mahanta[2]

Dacarbazine has shown the most consistent activity of any single chemotherapeutic agent in patients with metastatic melanoma. While the overall rate is 21%, responses fall to < 10% when hepatic metastases are present. We report a patient with malignant melanoma metastatic to the liver in whom an apparent dose-response relationship to dacarbazine was demonstrated. His liver metastases responded to hepatic artery infusion, progressed with systemic iv therapy, and responded upon reinstitution of hepatic artery infusion. [Cancer Treat Rep 70:1211-1212, 1986]

CASE REPORT

Ten years following enucleation of his left eye for malignant melanoma, a 60-year-old man presented to a local hospital with right upper quadrant abdominal pain. A chest x-ray disclosed a 6-cm right lower lobe lung mass and computerized axial tomographic (CAT) scan of the abdomen revealed possible liver metastases.

At thoracotomy, metastatic melanoma was found in the right posterior pleura and eighth rib; all detectable disease was resected. He was then referred to the St. Louis Veterans Administration Medical Center for further therapy. A postoperative assessment of the thorax by CAT scan and chest x-ray revealed no residual metastatic disease. After giving informed consent, the patient underwent laparotomy for confirmation of liver metastases and placement of a hepatic artery catheter.

Postoperatively, placement of the catheter was verified by means of contrast injection. The patient received 400 mg/m^2/day of dacarbazine (DTIC) in three divided doses by continuous hepatic artery infusion. This was given for 5 days every 3 weeks for three courses. The change in size of the index lesion in the liver in relationship to the administration of DTIC is shown in figure 1. Serial CAT scans revealed a decrease in the liver metastases but with the appearance of a new pulmonary nodule. In an attempt to control the pulmonary lesion, the fourth course of DTIC was given iv at a dose of 250 mg/m^2/day for 5 days as a 30-minute infusion. A CAT scan 3 weeks after the course of iv therapy revealed no interval change in the size of the pulmonary lesion but an increase in the size of the hepatic metastases; the abdominal pain had also worsened. Since the pulmonary lesion was stable and was causing no symptoms, subsequent courses of DTIC were given by hepatic artery infusion. Interval CAT scans following reinstitution of hepatic artery infusion of DTIC showed a reduction in the size of the hepatic metastases followed by stable disease (fig 1). Minimal progression of the pulmonary lesion was noted. The patient's abdominal pain improved and he experienced only mild nausea during administration of intra-arterial chemotherapy. At the time of manuscript preparation, the patient had received 13 courses of DTIC therapy. Rapid progression of the index lesion was observed during the last two courses of therapy and the DTIC was discontinued. Further intrahepatic chemotherapy with carmustine and cisplatin was initiated subsequently. The patient continued to do well with a Karnofsky performance status of 80% 18 months after the diagnosis of metastatic malignant melanoma.

DISCUSSION

The dose-response relationship of DTIC in metastatic melanoma was evaluated using the subject of this report as his own control. The high concentrations of DTIC delivered to the liver with intra-arterial therapy are augmented by hepatic extraction and metabolic activation of DTIC to active aminoimidazole carboxamide, thereby maximizing the amount of drug delivered to hepatic metastases. The drug concentration in the hepatic artery of our patient can be estimated using the drug infusion rate (0.58 mg/min) and the approximate rate of hepatic artery blood flow (200–600 ml/min). This calculation yields a range of 1–3 μg/ml. This value is only 1.4–4.5 times higher than concentrations achieved during 6-

[1] Received June 21, 1985; revised May 12, 1986; accepted May 23, 1986.

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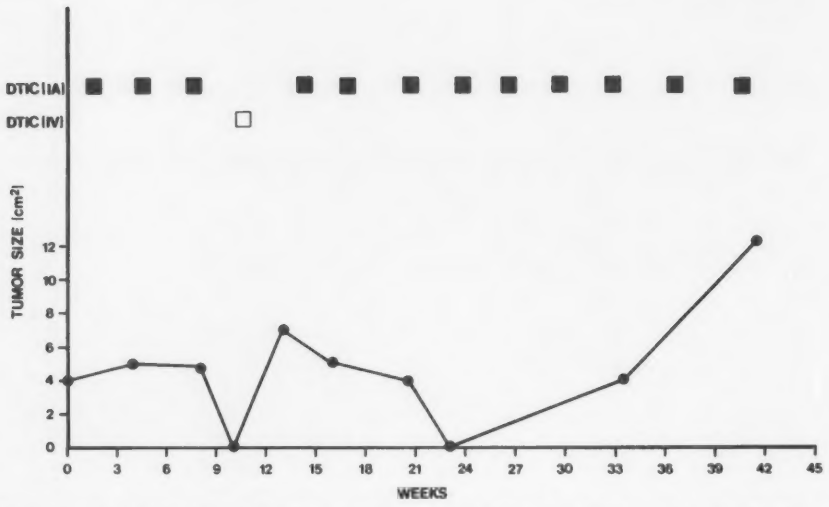

FIGURE 1.—Size of index liver metastasis as it relates to intrahepatic artery infusion and iv administration of DTIC.

hour iv infusions of DTIC, and much lower than the concentrations of DTIC achieved in the perfusate of isolated dog livers (1). However, hepatic artery infusion of DTIC at the rate and concentrations given to our patients over 5 days results in a much greater total dose delivered to the hepatic metastases.

A study performed by the Eastern Cooperative Oncology Group (2) did not verify a dose-response relationship in melanoma to DTIC given iv. However, the difference in doses was only twofold. Such differences are minimal when dealing with poorly responsive tumors and do not preclude a dose-response relationship when a wider range of doses is compared. A randomized study by the Central Oncology Group demonstrated an increased response rate with deliberate dosage escalation of DTIC given iv. However, durations of response and survival were not improved, and the number of patients treated was small (3).

We conclude that the melanoma in our patient demonstrated a dose-response relationship to DTIC. Generalizations cannot be made from a single case, including the use of hepatic artery infusion for melanoma. The infrequency of melanoma metastatic to the liver without concurrent extensive disease makes formal evaluation of this mode of therapy difficult.

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High-Dose Alpha-Tocopherol as a Preventive of Doxorubicin-Induced Alopecia[1]

Juan E. Perez,[2,*] Mario Macchiavelli,[3] Bernardo A. Leone,[4] Alberto Romero,[5] Mario G. Rabinovich,[4] Daniel Goldar,[6] and Carlos Vallejo[4]

The effectiveness of alpha-tocopherol acetate as a preventive of doxorubicin-induced alopecia was evaluated in 20 patients with different types of solid tumors. All received therapy containing doxorubicin in a dose range of 50–60 mg/m^2/cycle of treatment. The observed hair loss was severe in 90% and moderate in 10% of the patients. No protective activity of alpha-tocopherol was demonstrated in this trial. [Cancer Treat Rep 70:1213–1214, 1986]

Hair loss is a common and dreaded cutaneous side effect of antineoplastic chemotherapy. It is particularly notable for the anthracycline antibiotic doxorubicin, which usually induces total alopecia in nearly all patients. This drug has demonstrated an important activity against many tumors, but the profound social and psychological impact of hair loss causes some patients to refuse doxorubicin-based therapy.

Several studies have been carried out to find an efficient way to prevent this undesirable side effect. Scalp cooling is one of the most frequently used methods, although results to date are controversial (1–4). Recently, Wood (5) suggested the possible effectiveness of alpha-tocopherol as a preventive of doxorubicin-induced alopecia.

Encouraged by the promising results reported, we initiated a study aimed at confirming those findings in patients receiving doxorubicin at a dose > 40 mg/m^2/cycle of therapy.

PATIENTS AND METHODS

From June to October 1985, 20 female patients with different types of solid tumors were entered into this study. The median age was 53 years (range, 27–70). Diagnoses included breast carcinoma (17 patients), gastric carcinoma (two), and ovarian carcinoma (one). All patients were treated with combined chemotherapeutic regimens containing doxorubicin in a dose range of 50–60 mg/m^2 iv every 21–28 days. The following combinations were employed: doxorubicin and vincristine (AV) in seven patients; 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC) in five; doxorubicin and cyclophosphamide (AC) in five; doxorubicin and 5-fluorouracil (AF) in two; and cisplatin, doxorubicin, and cyclophosphamide (PAC) in one. A previous history of chemotherapy or radiation therapy to the cranium was the only exclusion criterion.

Starting 7 days before chemotherapy, all patients received alpha-tocopherol acetate orally at a dose of 1600 IU/day (400 IU every 6 hours). The vitamin was administered without interruption until hair loss developed, at which time tocopherol therapy was suspended. Patients were evaluated before each course of therapy and the following criteria were adopted: *Grade 1:* no hair loss; *Grade 2:* minor hair loss not requiring the use of a wig; and *Grade 3:* hair loss requiring the use of a wig.

RESULTS

The 20 patients entered into this trial were fully evaluable for response. Eighteen patients (90%) had Grade 3 alopecia, and two (10%) had Grade 2 alopecia. This effect was observed at the end of the first course of therapy in 50% of the patients; at the end of the second course in 40%; and after the third course in 10%. No toxicity attributable to alpha-tocopherol was observed.

DISCUSSION

Doxorubicin-induced alopecia is a distressing side effect causing a certain number of potential beneficiaries to refuse doxorubicin-based chemotherapy; therefore, an efficient way to prevent it would be welcomed.

The results obtained in this study using a slightly higher dose of doxorubicin than that reported by Wood (5) (50–60 mg/m^2 versus 40 mg/m^2) failed to demonstrate a significant activity of alpha-tocopherol as a preventive of doxorubicin-induced alopecia.

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While it is true that the combined regimens used in this trial included other drugs with a similar cutaneous effect, a more important protective role of tocopherol could be expected, since alopecia is principally induced by doxorubicin (6).

On the basis of our results, we do not recommend the use of high-dose alpha-tocopherol as a preventive of doxorubicin-induced alopecia.

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Cisplatin Administered as a Continuous 5-Day Infusion: Plasma Platinum Levels and Urine Platinum Excretion[1,2,3]

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Total and filterable platinum in plasma were monitored for seven courses (five patients, 25 mg/m²/day) using continuous 5-day infusions and one 30-minute infusion at a similar dose level (120 mg/m²). Maximum filterable (non-protein-bound) platinum levels (0.1–0.3 mg/L) for the extended infusions were ten to 40 times lower than that for the short-term infusion (4.0 mg/L). Filterable drug exposure as measured by the area under the $[Pt]_{filterable}$-time curve is greater for the extended infusions (9.6 mg·hr/L) than that for the short-term infusion (4.8 mg·hr/L). Renal excretion of cisplatin (% of dose excreted/24-hour period after the beginning of the infusion) was measured for four courses of continuous 5-day infusions and for the 30-minute infusion. Urine excretion rates were lower for the continuous infusion (5%–8% of dose/24-hour period during the infusion) compared to the short-term infusion (33% of dose/24-hour period after the beginning of the 30-minute infusion). [Cancer Treat Rep 70:1215–1217, 1986]

Infusion periods up to 24 hours using cisplatin have been investigated along with the measurement of blood/urine platinum parameters (1–9). Clinical studies of continuous 5-day infusion of cisplatin have appeared in the literature (10–12) without the corresponding blood/urine platinum parameters. The purpose of this study was to measure plasma platinum parameters and renal platinum excretion rates using continuous iv infusion of cisplatin.

METHODS

Five patients were treated with continuous 5-day infusions of cisplatin (25 mg/m²/3 L of saline/day) in the Division of Oncology, Roger Williams General Hospital. Preinfusion hydration was 500 ml of saline/2 hours and postinfusion hydration was 500 ml of saline with 20 mEq of KCl over 2 hours followed by 500 ml of 5% dextrose with 4 g of $MgSO_4$ over 2 hours.

Blood samples were drawn in heparinized tubes at 0, 3, 6, 24, 48, 72, 96, and 120 hours during infusion and 5, 30, and 60 minutes postinfusion. Urine samples were collected for each 24-hour period during infusion and for 24 hours postinfusion.

Unfiltered plasma was diluted fourfold with 10% HCl (vol/vol) and was analyzed directly for total platinum at 299.8 nm in a Spectraspan III (SMI/Beckman, Somerset, NJ), a conventional d-c argon plasma emission spectrometer. Calibration standards (0.2 and 2.0 mg/L) were made up in 10% HCl. Filterable (ie, non-protein-bound) platinum samples were obtained by centrifuging plasma samples at 1000 × *g* for 1 hour in CF50A Centriflo membrane cones (Amicon Corporation, Danvers, MA). In these samples, $[Pt]_{filterable}$ was analyzed at 299.8 nm

[1] Received Jan 15, 1986; revised Apr 29, 1986; accepted June 3, 1986.

[2] Supported by Public Health Service grants CA-20892 and CA-13943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and in part by Fellowship AIFCR No. 007 of the American-Italian Foundation for Cancer Research (LF).

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[10] We thank Mary Hillstrom, Lisa Launder, and Maribeth Donovan for their assistance in the platinum analyses and Ms. Brenda Gypson, MSN, and the nursing staff of West 4 Ward of Roger Williams General Hospital for their assistance in coordinating the collection of urine and blood samples.

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TABLE 1.—Pharmacokinetic parameters of plasma

Parameter	Present study*		Literature data†	
	5-day infusion	30-min infusion	< 2-hr infusion	24-hr infusion
Total dose (mg/m^2)	125	120	100	100
No. of patients	5	1	—	—
No. of courses	7	1	—	—
Maximum $[Pt]_{filterable}$ (mg/L)	0.138 (0.072–0.280)	4.04	1.4–9.0 (1–8)	0.27–0.39 (2,7)
Maximum $[Pt]_{total}$ (mg/L)	2.38 (1.55–2.72)	5.16	2.3–11 (2,4–6,8)	2.0–2.2 (2,9)
$[Pt]_{filterable}$ exposure (mg·hr/L)	9.6 (6.4–15.1)	4.8	1.8–8.3 (2,3,5)	5.4 (2)
$t_{1/2}$-$[Pt]_{filterable}$ (mins)	178 (40–835)	39	10–103 (1–4,7,13)	26 (2)

*Unless otherwise specified, values = average value (range).
†Unless otherwise specified, values = range (ref No.).

and calibration standards (0.10 and 0.20 mg/L) were prepared in 1:1 (vol/vol) saline:deionized water. Urine samples were analyzed in a manner similar to the filtrate samples using calibration standards of the appropriate platinum concentration range and saline content.

Plasma and urine samples from the patient receiving a 30-minute infusion were analyzed by utilizing identical procedures.

The natural logarithm of $[Pt]_{filterable}$ versus time data from the postinfusion phase was fitted to a single kinetic elimination term using a linear least-squares routine. This procedure gave estimates of the elimination rate constant (k_d), the half-life of elimination ($t_{1/2}$), and the plasma concentration (C_{max}) at the beginning of the postinfusion phase. The area under the $[Pt]_{filterable}$-time curve (AUC) was calculated using the trapezoidal rule during infusion and C_{max}/k_d for the postinfusion phase.

RESULTS

A comparison of plasma data for the 5-day infusion with those for the short-term infusion and literature data is given in table 1. The maximum filterable platinum levels for extended infusions (tenths of mgs/L) were ten to 40 times lower than the total platinum levels (mgs/L). The AUC exposure of free drug was greater than that for the short-term infusion at a similar dose level. For the patients on extended infusions, the elimination half-lives of filterable platinum in plasma had a very wide range compared to those reported in the literature. This wide range of half-lives may be artifactual because the measurements of the very low, filterable-Pt concentrations are complicated by postinfusion hydrations administered immediately after the continuous infusions of cisplatin. Our half-lives were evaluated using time points only during the 1-hour period after the end of the infusion. After the 1-hour postinfusion period, the platinum levels dropped to or below the detection limit of 20 μg/ml and had unacceptably large error limits. Thus, other plasma pharmacokinetic parameters, such as the volume of distribution and the total-body clearance, were not evaluated.

The renal drug excretion rates with extended infusions were 5%–8% in the 24-hour periods during and immediately after infusion as compared to 33% excretion for the 24-hour period following the short-term infusion at a similar total dose level.

DISCUSSION

The plasma data for continuous 5-day infusion of cisplatin observed in this study are in agreement with trends observed with shorter infusions (1–9). Maximum filterable platinum concentrations lowered from 2–9 mg/L for < 2-hour infusions to 0.3–0.4 mg/L for 24-hour infusions to 0.07–0.28 mg/L for 5-day infusions (table 1). This lowering of the maximum filterable platinum concentration may be related to the reduced nephrotoxicity, since urine clearance data showed that the rate of drug excretion is lowered as the infusion period is extended (2,4,13). Continuous-infusion cisplatin has proven minimally toxic in comparison with other maneuvers intended to improve the efficacy of this agent while controlling toxicity (10–12).

The AUC exposure of filterable platinum is greater with the longer infusion periods (table 1) and this potentially might improve efficacy compared to conventional bolus or short-term infusion treatments. Thus, the need for comparative clinical studies, including those with combination regimens, appears warranted.

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BRIEF REPORTS

Cisplatin and Etoposide Before Definitive Radiation Therapy for Inoperable Squamous Carcinoma, Adenocarcinoma, and Large Cell Carcinoma of the Lung: A Phase I–II Study of the Radiation Therapy Oncology Group[1,2]

James D. Cox,[3,*] Michael K. Samson,[4] Arnold M. Herskovic,[4] Ross Abrams,[5] and Maureen Koehler[5]

A trial of "neoadjuvant" cisplatin-etoposide and radiation therapy was conducted by the Radiation Therapy Oncology Group for non-small cell cardinoma of the lung limited to the thorax. Thirty evaluable patients were studied: two achieved complete response and four achieved partial response after chemotherapy. All patients underwent radiation therapy as planned, with no unusual acute reactions. Sixteen patients had local failure, and 13 had distant metastasis. Twenty-seven patients are dead, two are alive with cancer, and one is clinically free of cancer at 145 weeks. Five of six patients who survived ≥ 2 years after treatment had adenocarcinomas. There was no unexpected late toxicity. This combination of chemotherapy and radiotherapy is unlikely to improve results in the treatment of inoperable non-small cell carcinoma of the lung over those with radiotherapy alone. [Cancer Treat Rep 70:1219–1220, 1986]

Radiation therapy to the primary tumor and regional lymph nodes is the mainstay of treatment for patients with inoperable squamous carcinoma, adenocarcinoma, and large cell carcinoma of the lung. Investigators have noted antitumor activity of cisplatin in patients with non-small cell carcinoma of the lung (1,2). Etoposide has also been shown to have activity in cancer of the lung (3). Longeval et al (4) reported a 47% response rate in patients with non-small cell carcinoma of the lung with the combination of cisplatin and etoposide. These preliminary clinical results led to a prospective, phase I–II trial of the Radiation Therapy Oncology Group (RTOG).

METHODS

Patients were eligible if they had histologically confirmed, inoperable squamous carcinoma, adenocarcinoma, or large cell carcinoma of the lung, performance status ≥ 50 but no pleural effusion, ipsilateral supraclavicular lymphadenopathy, or superior vena caval obstruction. Prior radiation therapy and chemotherapy were criteria for exclusion. Adequate renal and normal hepatic functions and wbc count > 4000/mm^3 and platelet count ≥ 150,000/mm^3 were required. Patients were fully informed as to their diagnosis and the investigational nature of this study (written informed consent was obtained in accordance with standards of the Department of Health and Human Services and the member institutions). The participating institutions were the Medical College of Wisconsin Affiliated Hospitals and Wayne State University Medical School.

Cisplatin was administered at doses of 60 mg/m^2 iv on Days 1 and 22. Etoposide was given at doses of 120 mg/m^2 on Days 4, 6, 8, 25, 27, and 29. Full doses of chemotherapy were administered in the second and postirradiation courses if the weekly counts were ≥ 2000/mm^3 for the wbc count and ≥ 75,000/mm^3 for the platelet count. Lower nadir counts resulted in reductions of the doses of cisplatin and etoposide by 20%.

Megavoltage radiation therapy was started on the 42nd day.

The total dose was 50–60 Gy. Common fractionation was used, viz, 1.8–2.0 Gy five times each week to a volume which encompassed the primary tumor, the ipsilateral and contralateral hilar lymph nodes, the superior mediastinal and subcarinal lymph nodes, and the ipsilateral supraclavicular lymph nodes when the primary tumor presented in the upper lobe. The maximum dose permitted to the thoracic spinal cord was 45 Gy.

The usual criteria for response were used. Thoracic

[1] Received Dec 23, 1985; revised May 28, 1986; accepted June 13, 1986.
[2] Supported by Public Health Service grant CA-19565 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
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failure and distant metastasis were used as measures of the efficacy of combined chemotherapy and radiotherapy. Survival was calculated according to the actuarial (life-table) methods recommended by the American Joint Committee on Cancer (AJC).

PATIENTS

A total of 34 patients were entered in the study. Four patients were not evaluable: one had small cell carcinoma, two refused participation after being entered, and one had simultaneous cancer of the larynx. Age ranged from 32 to 73 years, with a median of 58 years. Male to female ratio was 24 to 6. Sixteen patients had squamous carcinoma, 12 had adenocarcinoma, and two had large cell carcinoma. Clinical stages (AJC) were I (three patients), II (one), and III (26).

RESULTS

Three patients received one course of chemotherapy and 27 received two courses. Two patients (one with squamous carcinoma; one with adenocarcinoma) had complete disappearance of all evidence of tumor, and four (three with squamous carcinoma; one with adenocarcinoma) had partial responses prior to radiation therapy.

Thirty patients received radiation therapy: four received total doses $\leq$ 50 Gy; six received between 53.2 and 54 Gy; and 20 received between 56.5 and 62.67 Gy.

Persistence or recurrence of the tumor within the field of irradiation occurred in 16 patients (53%); this was the first site of failure in 14 of them. Thirteen patients (43%) developed distant metastasis, of whom three also had progression within the field of irradiation.

The median survival for the 27 patients who have died was 52 weeks from the first day of chemotherapy (range, 8–148 weeks). Two patients are alive with evidence of cancer at 112 and 113 weeks. One patient is alive and clinically free of cancer of the lung at 145 weeks. The 1- and 2-year survival rates were 47% and 20%, respectively. Five of the six patients who lived $>$ 2 years had adenocarcinoma. No unexpected toxicity was encountered.

DISCUSSION

This study has confirmed the ability of combination chemotherapy with cisplatin and etoposide to produce a small number of complete responses in patients with squamous carcinoma and adenocarcinoma. Sculier and Klastersky (5) reviewed the studies of cisplatin plus etoposide and/or vindesine in non-small cell carcinoma of the lung. They noted that 34 of 762 patients (4.5%) had complete response. In the RTOG study, cisplatin was administered at a dose of 60 mg/m^2. In the review noted above, there was no difference in complete response rates among patients who had received cisplatin at doses $\leq$ 100 mg/m^2 compared with those who received doses $\geq$ 100 mg/m^2.

The RTOG has conducted a number of studies of patients with inoperable carcinoma of the lung. The survival and failure patterns in this study were similar to RTOG studies which have used radiation therapy alone in a similar patient population (6). Therefore, there are no plans to use this combination of chemotherapy and radiation therapy in future RTOG trials for cancer of the lung.

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BRIEF REPORTS

Phase II Evaluation of Bolus Gallium Nitrate in Lymphoproliferative Disorders: A Southeastern Cancer Study Group Trial[1,2]

James Keller,[3] Alfred Bartolucci,[4] John T. Carpenter, Jr,[4] and John Feagler[5,*]

Gallium nitrate was administered as a 700-mg/m² iv bolus infusion over 15–30 minutes every 2 weeks to 138 patients with malignant lymphoproliferative diseases. Responses occurred in patients with well-differentiated lymphomas (five responses among eight patients), but the drug produced few responses in any other group of patients. Toxic effects were primarily gastrointestinal and reversible renal abnormalities and anemia. As a single agent, bolus gallium nitrate has little activity in lymphoproliferative diseases. [Cancer Treat Rep 70:1221–1223, 1986]

Gallium nitrate is an inorganic heavy metal compound that has appeal as a chemotherapeutic agent because it lacks significant myelotoxicity. However, like cisplatin, another inorganic metal compound, it has renal toxicity. In 1980, the Southeastern Cancer Study Group (SEG) undertook a phase II trial of this agent in lymphoproliferative disorders, ie, Hodgkin's disease, non-Hodgkin's lymphomas, and chronic lymphocytic leukemia. Initial phase I studies (1–4) prompted us to use a bolus dose of gallium nitrate (700 mg/m² every 2 weeks) for this study.

PATIENTS AND METHODS

One hundred thirty-eight patients were entered in this protocol. Eligibility requirements included a histological diagnosis, relapse after first- and second-line chemotherapy, measurable lesion, Karnofsky performance status > 40%, recovery from the effects of previous therapy including corticosteroids, creatinine clearance > 60 ml/minute, serum creatinine < 1.5 mg/dl, bilirubin < 1.2 mg/dl, life expectancy > 6 weeks, age > 18 years, absence of congestive heart failure, and signed informed consent. Patients were stratified as follows: Hodgkin's disease, favorable (nodular poorly differentiated, nodular mixed, and well-differentiated) and unfavorable (diffuse poorly differentiated, diffuse mixed, nodular histiocytic, and diffuse histiocytic); non-Hodgkin's lymphomas; and chronic lymphocytic leukemia. Histologic diagnosis according to the Rappaport classification was accepted from the submitting institution.

A course of therapy consisted of gallium nitrate (700 mg/m² in 100 ml of 5% dextrose in water) infused iv over 15–30 minutes. Patients were hydrated with 1–2 L of fluid orally or iv in the 6–12 hours before treatment and 2 L of fluid over the next 24 hours. Mannitol (12.5 g) was given iv just prior to the gallium nitrate infusion. Subsequent courses of treatment were given every 2 weeks. Evaluation of response and toxicity was made 2 weeks after the second course. Responding patients and those showing no progression were continued on therapy until their disease progressed.

Toxicity was assessed according to SEG criteria (5). Patients achieved complete remission if there was a complete resolution of all measurable disease and partial remission if there was at least a 50% reduction in the products of the diameters of measurable disease for at least 1 month.

[1] Received July 8, 1985; revised May 28, 1986; accepted June 26, 1986.

[2] Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Alabama at Birmingham (CA-03013); Southeastern Cancer Study Group Statistical Center, Birmingham, AL (CA-24456); Southeastern Cancer Study Group, Birmingham, AL (CA-19657); Duke University School of Medicine, Durham, NC (CA-03177); Emory University School of Medicine, Atlanta, GA (CA-03227); University of Miami School of Medicine, FL (CA-05641); Temple University School of Medicine, Philadelphia, PA (CA-07962); Washington University School of Medicine, St. Louis, MO (CA-03376); University of Puerto Rico, San Juan (CA-12223); University of Tennessee Memorial Research Center, Knoxville (CA-13237); Case Western Reserve University School of Medicine, Cleveland, OH (CA-15584); University of Tennessee School of Medicine, Memphis (CA-17027); St. Louis University School of Medicine, MO (CA-17214); University of Kentucky, Lexington (CA-20255); University of Florida School of Medicine, Gainesville (CA-28143); Indiana University School of Medicine, Indianapolis (CA-28171); and City of Hope Medical Center, Duarte, CA (CA-30247).

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TABLE 1.—Patient characteristics, response data, and toxicity*

Characteristic	
Median age in yrs (range)	57.1 (18–81)
Median performance status (range)	80% (40%–100%)
Prior treatment	
Chemotherapy only	
Hodgkin's disease	9
Non-Hodgkin's lymphoma	11
Chronic lymphocytic leukemia	9
Chemotherapy + radiotherapy	
Hodgkin's disease	22
Non-Hodgkin's lymphoma	47
Chronic lymphocytic leukemia	7
Unknown	
Chronic lymphocytic leukemia	1
Stage (excluding chronic lymphocytic leukemia)	
II	2
IIE	3
III	8
IIIE	1
IIISE	2
IV	95
Unknown	2
Response to therapy (partial response/no progression)	
Hodgkin's disease ($n = 31$)	4/10
Favorable non-Hodgkin's lymphoma	
Nodular poorly differentiated lymphoma ($n = 14$)	0/6
Nodular mixed lymphoma ($n = 3$)	0/1
Well-differentiated lymphoma ($n = 8$)	5/1
Unfavorable non-Hodgkin's lymphoma	
Diffuse poorly differentiated lymphoma ($n = 11$)	1/3
Diffuse mixed lymphoma ($n = 6$)	1/1
Nodular histiocytic lymphoma ($n = 5$)	1/1
Diffuse histiocytic lymphoma ($n = 36$)	1/4
Chronic lymphocytic leukemia ($n = 17$)	0/0
Toxicity	
Granulocytopenia (cells/μl)	
1000–1900	10
< 1000	2
Thrombocytopenia (cells/μl)	
90,000–130,000	11
Anemia (hemoglobin, 8.5–11 g/dl)	43
Infection	6
Renal (creatinine clearance)	
< 50% reduction	23
≥ 50% reduction	4
Hepatic (SGOT or alkaline phosphatase)	
< 5 times normal value	11
≥ 5 times normal value	3
Nausea and vomiting (controllable)	18
Diarrhea	20
Stomatitis	4

*Unless otherwise specified, values = No. of patients.

RESULTS

One hundred thirty-eight patients were entered in this protocol. Seven patients were not eligible (two had renal insufficiency, two had incorrect histology, and three died of disease progression during the first 14 days in the study before evaluation of response could be made). Responses are shown in table 1. There were no complete responses. Patients in the group with most responses had well-differentiated lymphomas (five responses among eight patients). A few partial responses were also seen in Hodgkin's disease and some unfavorable non-Hodgkin's lymphomas. Responses were generally of short duration (1–23 months, median, 3.3).

Toxicity (table 1) was mild and consisted primarily of hematopoietic, gastrointestinal, and reversible renal abnormalities. Metallic taste was recorded in a few patients but its presence was not routinely inquired about. Hearing loss, superimposed on chronic loss, was recorded in one instance. Hypocalcemia was encountered in four patients, and in all it was associated with some rise in serum creatinine. Hypomagnesemia, a known complication of gallium, was reported in one patient. Renal tubular acidosis was reported in two patients but not well-documented. Confusion and lethargy were observed in several patients; all symptoms improved in a matter of weeks and further gallium was not given. One patient developed tonic flexion posturing of the head on the chest without apparent cause which was refractory to treatment with Cogentin, phenothiazines, and correction of electrolytes.

DISCUSSION

Treatment of malignant lymphoproliferative diseases with bolus gallium nitrate in this study was ineffective with the exception of the well-differentiated lymphocytic lymphomas. Weick et al (6) reported the experience of the Southwest Oncology Group (SWOG) with bolus gallium nitrate using the same dosage schedule. Their response rate was 18% (seven responses among 38 patients) with no responders in well-differentiated lymphomas (no responses among four patients). We have no explanation for the disparity between the response rates observed in patients with well-differentiated lymphocytic lymphomas in the two studies or between those and the response rates seen in patients with chronic lymphocytic leukemia, except to note that the total number of patients with such lymphomas is quite small (total, 12). Warrell et al (7) recently reported their results with gallium nitrate at Memorial Sloan-Kettering, administered as a continuous infusion of 300 mg/m^2/day for 7 days every 3–5 weeks. Responses were seen in diffuse histiocytic lymphoma (six responses among 15 patients; two complete); diffuse poorly differentiated lymphoma (five responses among ten patients); nodular poorly differentiated lymphoma (two responses among five patients); and Hodgkin's disease (three responses among 17 patients) (34% overall).

From the analysis of the SEG, SWOG, and Memorial Sloan-Kettering data, one could tentatively conclude the following, realizing that small numbers of patients were studied and that histologic classification may be inaccurate because of transformations and institutional variations in pathologic interpretation. (*a*) Bolus gallium therapy appears ineffective in chronic lymphocytic leukemia. (*b*) With the exception of well-differentiated lymphomas in this study, bolus gallium therapy is ineffective (< 20%

response rate) in Hodgkin's disease, as well as in favorable and unfavorable non-Hodgkin's lymphomas. (*c*) Continuous-infusion gallium may be more effective and deserves further study in patients with lymphoproliferative diseases.

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BRIEF REPORTS

Central Nervous System Toxicity of Fludarabine Phosphate[1]

Hoo G. Chun,* Brian R. Leyland-Jones, Sophia M. Caryk, and Daniel F. Hoth[2]

Fourteen patients developed severe central nervous system (CNS) toxicity after receiving an investigational antitumor agent, fludarabine phosphate (FAMP). The CNS toxicity has the distinctive features of delayed onset and progressive clinical course. Visual deficits were the most common presenting symptom and developed eventually in most cases. Deterioration of mental status and progressive encephalopathy were also observed. The development of clinical CNS toxicity appears dose-related; thirteen of 36 patients (36.1%) who received FAMP at high doses ($\geq$ 96 mg/m^2/day for 5–7 days per course) developed neurotoxicity, while only one of 443 patients (0.2%) who received the drug at low doses ($\leq$ 125 mg/m^2 per course) developed similar toxicity. Although the precise mechanism responsible for this toxicity is yet unknown, progressive demyelination appears to be the responsible process. Extensive review of the clinical data failed to identify factors which might contribute to the development of CNS toxicity. Patients on trials of FAMP should be meticulously monitored for the possible development of neurotoxicity. [Cancer Treat Rep 70:1225–1228, 1986]

Fludarabine phosphate (2-fluoro-ara-AMP; FAMP) is a new purine nucleoside antitumor agent that has recently entered clinical trials. Its mechanism of action for cytotoxicity appears related to the inhibition of DNA synthesis by its phosphorylation to the triphosphate form, fluoro-ara-ATP. The agent has antitumor activity against several murine tumors and human xenografts. The principal target organ for toxicity in animals was bone marrow, and gastrointestinal toxicity was also observed (1). The dose-limiting toxicity was myelosuppression during phase I trials in patients with solid tumors. Other toxic effects included nausea and vomiting, elevation of liver enzymes, fatigue, and transient somnolence (2–4). During phase I/II trials in acute leukemias and phase II trials in solid tumors, incidents of severe central nervous system (CNS) toxic effects have been encountered. We describe here the details of CNS toxicity seen in 14 patients and attempt to elucidate the nature of and possible mechanisms underlying this toxicity.

MATERIALS AND METHODS

The patients described were reported to the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute (NCI), between October 1984 and November 1985, after being treated with FAMP according to either one of the five phase I/II leukemia or 27 phase II solid tumor protocols. Clinical data of all patients entered on the leukemia trials were reviewed by the NCI, with special attention to prior therapy and other factors predisposing for CNS toxicity. Summaries of clinical data of patients on solid tumor trials were obtained after being reviewed either by the principal investigator or the study chairperson of the protocol for possible neurotoxicity.

RESULTS

A total of 70 patients (ten children) with acute leukemia received 95 courses of FAMP at daily doses ranging from 20 to 220 mg/m^2 for 5–7 days by either short ($\leq$ 1 hour) or continuous infusion. No incidents of clinical CNS toxicity were observed among 34 patients who received the drug at daily doses of $\leq$ 80 mg/m^2 for 5 days. Of 36 patients who received at least one course of FAMP at daily doses of $\geq$ 96 mg/m^2 for 5–7 days, 13 experienced severe CNS toxicity. Their characteristics and clinical data are summarized in table 1. Five patients had received prior high-dose (HD) cytarabine (ara-C) and four had received prior intrathecal chemotherapy (ITC) with either methotrexate or ara-C, or both. Two patients had a residual neurologic deficit from prior therapy at the time of entry to the FAMP trial; Patient 9 had decreased mentality with slow speech after HD ara-C and Patient 10 had peripheral neuropathy after

[1] Received Dec 13, 1985; revised May 22, 1986; accepted June 26, 1986.
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TABLE 1.—Summary of clinical information on 13 patients

Patient No.	Age (yrs)/ sex	Disease	Daily dose of FAMP (mg/m^2)	Schedule	Prior treatment			Neurotoxicity	
					HD-AC	IT	WBRT	Onset (Day)†	Presentation/progression
1	66/M	ANLL	100 125	D × 5	+	–	–	– 26	None Spastic paraparesis/confusion, loss of vision, encephalopathy
2	49/F	ANLL	100 125	D × 5	+	–	–	– 26	None Spastic paraparesis/drowsiness, sensory loss in legs, loss of vision, encephalopathy
3	24/F	CML-BC	125	D × 5	+	–	–	39	Paresthesia in extremities/urinary incontinence, sensory loss, loss of vision, coma
4	27/M	ANLL	150	CI × 5D	–	–	–	29	Photophobia/confusion, change in behavior, hallucination, loss of vision, seizure, semicoma, coma
5	47/F	ANLL	125	CI × 7D	–	–	–	41	Blurred vision/cortical blindness, paraparesis, quadriparesis, mild encephalopathy → gradual but complete neurologic recovery
6	27/F	ANLL	150	CI × 5D	–	–	–	34	Bilateral papillitis/altered mental status, incontinence, loss of vision, encephalopathy, coma
7	28/F	ALL	125	CI × 7D	–	MTX	–	21	Dysphasia/lethargy, ataxia, loss of vision, obtundation, coma
8	49/M	ANLL	125	CI × 7D	–	–	–	43	Generalized seizure/lethargy, respiratory arrest
9	57/M	ANLL	77 96	D × 5	+	–	–	 24	None Confusion and dementia/coma
10	32/M	ANLL	100	CI × 5D	–	–	–	45	Loss of vision (optic neuritis)/change in mental status, urinary incontinence, spastic extremities, encephalopathy
11	2/F	ANLL	125 150	CI × 5D	–	MTX + AC	–	 60	None Blurred vision/encephalopathy
12	7/F	ALL	150 175	CI × 5D CI × 5D	–	MTX	–	 25	None Diplopia/slurred speech, loss of head control, cortical blindness, encephalopathy
13	6/F	ANLL	150 220	CI × 5D CI × 5D	+	AC	–	 22	None Loss of vision/encephalopathy, coma

*HD-AC = high-dose cytarabine; IT = intrathecal treatment; WBRT = whole-brain radiotherapy; CT = computerized tomography; MBP = myelin-INC = increased; ND = not done; CML-BC = blastic phase of chronic myelogenous leukemia; CI × 5-7D = continuous infusion for 5–7 days; CR = MLE = multifocal leukoencephalopathy; and AC = cytarabine.

†Days from the initiation of the last course of FAMP administration.

‡Values = ng/ml.

vincristine and ara-C. Meanwhile, among 23 patients who received similar HD FAMP (≥ 96 mg/m^2/day for 5–7 days) but without clinical CNS toxicity, three patients had received prior HD ara-C, eight had received prior ITC, and two had received prior brain irradiation. Therefore, neither prior HD ara-C nor previous ITC appears to be a predisposing factor for the development of CNS toxicity after FAMP.

The onset of neurologic symptoms or signs was delayed in all cases; they appeared 21–60 days after receiving the last course of FAMP. Visual symptoms, seen in seven patients, were the most frequent presenting symptom, and loss of vision eventually occurred in 11 patients. Of note, myelopathic clinical pictures were the presenting symptoms in three patients who received the drug by short infusion. Progressive deterioration of mental status or encephalopathy leading to a vegetative state developed in 11 patients. Eleven patients underwent a computerized tomographic scan of the brain, which showed no specific abnormal findings except for some degree of cortical atrophy in two patients and a suspicious decrease of white matter in paraventricular areas in Patient 5. Nuclear magnetic resonance (NMR) scans of the two patients (Nos. 5 and 6) showed an

with CNS toxicity after administration of FAMP*

Diagnostic studies							
	Cerebrospinal fluid						
CT	Glucose	Protein	MBP‡	Cytology	EEG	Autopsy	Outcome
WNL	WNL	INC	ND	—	Diffuse slowing	Demyelination in spinal cord	Died on Day 56
WNL	INC	INC	15	—	Diffuse slowing	ND	Died on Day 46
WNL	WNL	WNL	17	—	Diffuse slowing	Demyelination in cerebral cortex and spinal cord	Died on Day 55
WNL	INC	WNL	ND	—	Diffuse slowing	ND	CR, but died on Day 67 with relapse
ABN	WNL	INC	9	—	ND	ND	CR, but died on Day 284 with relapse
WNL	WNL	WNL	ND	—	High-voltage irregularity	ND	CR, but died on Day 139 with relapse
WNL	WNL	INC	ND	—	ND	ND	CR, but died on Day 73 with remission
CA	ND	ND	ND	ND	ND	Anoxic brain damage	Died on Day 53 of respiratory failure
WNL	NA	NA	NA	—	ND	MLE	Died on Day 65
WNL	NA	INC	INC	NA	NA	Demyelination in occipital lobe with axonal loss	CR, but died on Day 89 with relapse
CA	ND	ND	ND	ND	ND	MLE	Died on Day 100
ND	NA	NA	NA	—	ND	ND	Died on Day 67
ND	ND	ND	ND	ND	ND	ND	Died on Day 42

basic protein; EEG = electroencephalography; ANLL = acute nonlymphocytic leukemia; D × 5 = daily for 5 days; WNL = within normal limit; complete remission; ABN = abnormal; ALL = acute lymphoblastic leukemia; MTX = methotrexate; CA = cortical atrophy; NA = not available;

extensive diffuse loss of white matter. Analyses of CSF from ten patients revealed an elevated protein level in five and negative cytologic examination in all. The myelin-basic protein level was elevated in CSF from four patients examined. Abnormal electroencephalographic study was reported in all five patients tested. Visual-evoked potentials were absent in the three patients (Nos. 5, 6, and 10).

All 13 patients died. Although five patients achieved complete remission, the remission duration was short (22, 30, 40+, 60, and 71 days) and only one patient (No. 7) was in an unmaintained remission at the time of death. Postmortem examination of the CNS revealed various degrees of demyelination, frequently multifocal or diffuse, in the brain and spinal cord (especially in the occipital cortex, around the optic tracts) in five patients. A diagnosis of multifocal leukoencephalopathy was reported in two cases. One patient (No. 5) had gradual recovery of neurologic symptoms, including loss of vision, over a several-month period, but died of relapsed acute nonlymphocytic leukemia.

Similar CNS toxicity has been observed in a patient who received courses of a "low" dose of the drug. A 66-year-old male with mycosis fungoides, with a past his-

tory of mumps encephalitis, received three courses of FAMP at 18–22.5 mg/m^2/day for 5 days. On Day 20 after the third course, the patient presented with a transient episode of weakness, somnolence, disorientation, and visual hallucinations. Decreased memory, disorientation, spastic extremities, and an episode of right-sided Bell's palsy developed over the next several weeks without further treatment with FAMP. On Day 96 after the third course of FAMP, his vision began to deteriorate with recurrence of hallucination and disorientation. With the development of complete blindness and progressive encephalopathy, his condition deteriorated with episodes of sepsis; he was comatose for several days prior to death on Day 137 after the third course of FAMP. Electroencephalography, visual-evoked response test, and NMR with series of computerized tomographic scan of the brain during this period were unremarkable. The CSF protein levels were elevated up to 153 mg/dl, but with undetectable myelin-basic protein levels and negative cytology. On autopsy, microscopic examinations disclosed lymphomatous infiltration of the cerebellopontine area consistent with mycosis fungoides. In addition, focal severe myelinolysis involving the optic tracts, the basis pontis, and the cerebral peduncles was found.

As of October 1985, 443 patients have been entered onto 27 phase II solid tumor trials. The dose and schedules used are either by short infusions as a single dose of 125 mg/m^2, as 18–30 mg/m^2/day for 5 days, or as 15–20 mg/m^2 every 12 hours for 6 doses, or by continuous infusions of 20 mg/m^2/day for 5 days or 30 mg/m^2/day for 48 hours with a loading dose of 20 mg/m^2. The treatments were repeated at 3–4-week intervals. Among 202 patients for whom information on repeated treatment with FAMP is available, 42 have received three or more courses of the drug. No patient other than the one described above had any significant neurologic problem considered to be related to FAMP.

DISCUSSION

Fourteen cases of CNS toxicity with the distinctive features of delayed onset and progressive clinical course after receiving FAMP are described. While 36.1% of the patients who received FAMP at high doses ($\geq$ 96 mg/m^2 daily for 5–7 days per course) developed CNS toxicity, only 0.2% of patients who received the drug at low doses ($\leq$ 125 mg/m^2 per course) developed the toxicity. No incidents of similar CNS toxicity have been observed among 34 patients with acute leukemias who received the drug at intermediate doses (20–80 mg/m^2 daily for 5 days). Therefore, the clinical CNS toxicity appears to be dose-related. Its relationship to either peak plasma level or cumulative doses of FAMP is uncertain, since clinical toxicity developed on schedules of both short (bolus) and continuous infusion and since no other incidents have been seen in patients who received repeated low doses of FAMP with a cumulative dose equivalent to high-dose schedules. Despite extensive review of the clinical data, factors which might contribute to this toxicity have not been identified.

Although individual clinicopathologic findings are somewhat variable, progressive demyelination in the CNS appears to be the responsible process which results in, or at least contributes to, the fatal outcome in all patients but one. The precise mechanism responsible for the demyelination after FAMP is unknown. Further investigation, including in vitro and animal studies, is obviously necessary for a better understanding.

FAMP may be effective for remission induction in both acute nonlymphocytic leukemia and acute lymphoblastic leukemia. However, it is unfortunate that the drug has little or no therapeutic index in this situation, ie, there is a high incidence of unacceptable CNS toxicity at doses required to achieve remission (5). Patients on low-dose trials should be meticulously monitored, with frequent neurologic evaluation, serial NMR scan, and visual-evoked response test.

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High-Dose Cisplatin in the Treatment of Advanced Adenocarcinoma of the Colon and Rectum: A Southeastern Cancer Study Group Trial[1,2]

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Cisplatin at a dose of 50 mg/m^2 has not been active in colon cancer (1), but the possibility of activity at higher doses remained to be evaluated. The following is the experience of the Southeastern Cancer Study Group using high-dose cisplatin (120 mg/m^2) in patients with previously treated and untreated colorectal adenocarcinoma.

Adults were eligible for entry into the study if they had histologic confirmation of disease (residual, recurrent, or metastatic); gave written informed consent; were ambulatory with an estimated survival of 6 weeks; had an easily measurable lesion; had recovered from previous myelosuppressive therapy; had an adequate bone marrow reserve; and had serum creatinine < 1.2 mg/dl. All patients were stratified into three groups: no prior chemotherapy, minimal prior chemotherapy (one drug), and extensive prior chemotherapy. A partial response was defined as a decrease $\geq 50\%$ in the sum of the products of the diameter of measurable lesions and/or a reduction by 30% of the sum of liver measurements below the costal margin. Toxicity was defined according to standard criteria (2).

Cisplatin, 120 mg/m^2, in 250 ml of saline was infused iv over 60 minutes every 3 weeks. All patients received the following hydration protocol: 6 hours prior to receiving cisplatin they were given 5% dextrose in one-half normal saline at 150 ml/hour with 10 mEq of KCl; cisplatin was immediately followed by 6 hours of normal saline at 150 ml/hour and 50 g of mannitol. All patients were to have received at least two courses of treatment to be evaluable for response.

Table 1 reveals there were four responders (all with liver involvement): two (partial responses) in the group of patients who had had no prior treatment and two (one complete response and one partial response) in the group who had received only one prior agent (5-fluorouracil).

TABLE 1.—Patient characteristics*

Characteristic	Prior chemotherapy		
	None	1 Agent	Extensive
Entered	31	15	8
Evaluable	31	14	8
Sex			
Men	25	7	2
Women	6	7	6
Mean age (yrs)	59	59	63
Mean Karnofksy performance status (%)	99	80	70
No. of responses			
Complete	—	1	—
Partial	2	1	—

*Unless otherwise indicated, values = No. of patients.

No responses were noted in the group who had received extensive prior therapy. The response rate among all evaluable patients was 9% (four of 53 patients).

As expected, the major toxic effect was nausea and vomiting, with 77% of the patients having grade II reactions controllable with antiemetics. Nine patients had nadir granulocyte counts $< 1000/\mu l$ and four had nadir platelet counts $< 50{,}000/\mu l$. Patients experiencing renal toxicity included 17 with creatinine of 1.3–2.0 mg/dl, six with creatinine between 2.1 and 3.0 mg/dl, and one patient who developed renal failure that was reversible. No patient was noted to have a hearing loss.

We conclude that high-dose cisplatin as a single agent has only minimal activity in advanced colorectal cancer. Whether this drug is synergistic with 5-fluorouracil in colon cancer, as suggested by a recent report (3) of a

[1] Received Jan 7, 1986; revised Apr 7, 1986; accepted Apr 14, 1986.

[2] This study was performed by the Southeastern Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Alabama at Birmingham (CA-03013, CA-19657, CA-24456); Duke University, Durham, NC (CA-03177); Emory University, Atlanta, GA (CA-03227); Temple University, Philadelphia, PA (CA-07961); Washington University, St. Louis, MO (CA-03376); University of Puerto Rico, San Juan (CA-12223); University of Tennessee, Knoxville (CA-13237); Case Western Reserve University, Cleveland, OH (CA-15584); St. Louis University, MO (CA-17214); Indiana University, Indianapolis (CA-28171); University of Virginia, Charlottesville; University of Iowa, Iowa City (CA-37577); University of Miami, FL (CA-05641); Vanderbilt University, Nashville, TN (CA-23909); Ochsner Clinic, New Orleans, LA.

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29% response rate for the combination, remains to be determined.

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Teniposide in Epithelial Ovarian Carcinoma: A Phase II Trial of the Gynecologic Oncology Group[1,2]

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Teniposide (VM-26) and etoposide (VP-16) are semisynthetic products derived from podophyllotoxin which cause metaphase arrest; unlike vinca alkaloids such as vincristine, however, they do not bind to mitotic spindle proteins (1). Numerous phase I and II trials with VM-26 (2) have indicated activity in many sites but there have been few reports in gynecologic malignancy. Samson et al (3) treated 16 previously treated patients with

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	GOG performance status 0–4	Radiation therapy	Prior chemotherapy*	No. of courses	Response†	Progression-free interval (mos)	Survival (mos)
1	38	1	No	CAP	7	ID	1.6	1.7+
2	45	1	Yes	CAP, XRT	1	ID	3.3	3.3
3	45	1	No	CAP	6	ID	1.7	1.7+
4	46	2	No	ADR-CIS	3	ID	1.4	3.7
5	50	2	No	CAP	2	ID	.6	.6
6	50	0	Yes	CAP, XRT, TAMOX	4	ID	.9	5.7
7	51	0	No	CAP-BCG	5	ID	2.2	4.6
8	52	0	No	CAP-BCG	3	ID	1.1	1.5
9	52	1	No	CAP	7	ID	2.3	2.3+
10	53	0	No	CAP	9	SD	2.1+	2.1+
11	55	1	No	CAP	11	PR	3.6	3.7+
12	56	1	Yes	CAP, XRT	3	ID	.5	1.7
13	57	1	No	CAP	2	ID	.5	.5
14	58	1	No	CIS-CTX	4	NE	3.4	3.4
15	58	2	No	CAP	2	NE	1.3	1.3
16	60	0	No	CAP, TAMOX	6	PR	3.7	4.0
17	60	2	No	CAP-INTERFERON	3	ID	1.1	3.3
18	61	0	No	CAP	6	ID	1.8	8.2+
19	61	1	Yes	CAP, XRT	16	SD	4.1+	4.1+
20	62	2	No	CAP	18	SD	4.4+	4.4+
21	63	1	No	CAP	5	ID	1.6	1.8+
22	63	0	No	CAP	15	SD	3.7	3.7+
23	67	0	No	CAP	2	ID	.5	.5
24	68	2	No	CAP	6	ID	2.3	2.9
25	68	1	No	CAP	13	PR	5.9	5.9+
26	69	1	No	CTX	14	SD	3.8+	3.8+
27	72	1	No	CIS-ADR	7	SD	3.0	4.5
28	74	1	No	CTX-CIS	2	ID	.6	2.8

*CAP = cyclophosphamide, doxorubicin, and cisplatin; XRT = radiation therapy; ADR-CIS = doxorubicin + cisplatin; TAMOX = tamoxifen; BCG = bacillus Calmette-Guérin; and CTX = cyclophosphamide.

†ID = increasing disease; SD = stable disease; PR = partial response; and NE = not evaluable for response.

[1] Received Dec 16, 1985; revised Apr 10, 1986; accepted Apr 17, 1986.

[2] Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: University of Oregon Health Sciences Center, Portland; Hahnemann Medical College, Philadelphia, PA; Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA; Temple University School of Medicine, Philadelphia, PA (CA-12478); University of Minnesota Medical School, Minneapolis (CA-23088); Colorado Foundation for Medical Care, Denver (CA-15975); Georgetown University Hospital, Washington, DC (CA-16938); The University of Texas Health Science Center at Dallas (CA-28160); Indiana University Medical Center, Indianapolis (CA-21720); The Albany Medical College of Union University, NY; University of California Medical Center at Irvine (CA-23765); Illinois Cancer Council Comprehensive Cancer Center, Chicago (CA-27806); St. Louis University Medical Center, MO (CA-35571); and Jacksonville University Hospital, FL.

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ovarian cancer with 20–30 mg/m^2 of VM-26 daily for 5 consecutive days every 3–4 weeks without noting response. VP-16, however, was found to have a 12% complete and partial response rate in 101 ovarian cancer patients, of whom most had been previously treated (4).

Eligibility required measurable disease, no more than one prior chemotherapy regimen, Gynecologic Oncology Group (GOG) performance status of 0–2 (Zubrod, Eastern Cooperative Oncology Group), adequate hematologic and hepatic function, and informed consent. The starting dose and schedule was 100 mg/m^2/week administered as a 30–60-minute infusion. Patients without toxicity had escalations of 20 mg/m^2/week until a maximum dose of 160 mg/m^2 was reached. Dose modifications were made for hematologic or gastrointestinal toxicity. For patients with lesions demonstrable only on computerized tomographic, sonic, or radionuclide scan, repeat studies were done at 6-week intervals. Palpable lesions were assessed every 3 weeks and standardized GOG criteria (5) were used to assess response. An adequate trial of therapy was defined as receiving one dose of drug and alive at 4 weeks. All patients who received at least one dose of VM-26 were considered evaluable for response and toxicity.

Pretreatment characteristics for each patient are shown in table 1. Thirty-two patients were entered and four were excluded. Two additional patients could not be included for response assessment since they were removed early in the study due to toxicity, notably seizures in one (probably due to cerebral infarct) and bronchospasm in another.

Three partial responses, all in palpable lesions, were observed in 26 evaluable patients, with progression-free intervals of 3.6–5.9 months. Three patients developed severe neutropenia (granulocyte count $< 500/mm^3$), fever, and presumed sepsis, and all recovered. Mild nausea or vomiting was seen in six patients. The response rate to VM-26 was 12% (three responses among 26 patients) and the 95% confidence interval for response was 2.4%–30%. Toxicity was moderate. These data support only modest activity for VM-26 in previously treated patients with epithelial ovarian cancer.

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Phase II Trial of *m*-AMSA Therapy in Refractory Hodgkin's Disease and Non-Hodgkin's Lymphoma: Cancer and Leukemia Group B Study 7972[1,2]

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m-AMSA is an antitumor agent which inhibits DNA synthesis. The major toxic effect is myelosuppression; other side effects include nausea and vomiting, increase in serum alkaline phosphatase, allergic reactions, and cardiotoxicity.

Reported activity of *m*-AMSA in relapsed and refractory lymphoma has ranged from 15% to 45% (1-4). This phase II study was carried out to better define the frequency of responses to *m*-AMSA in refractory Hodgkin's disease and non-Hodgkin's lymphoma and the duration of response on continued *m*-AMSA therapy.

PATIENTS AND METHODS

Patients admitted to this study had non-Hodgkin's lymphoma or Hodgkin's disease refractory to one or more drug therapies. Before entry, patients were required to have histologically documented and restaged lymphoma, measurable disease, and projected survival >2 months. Any patient performance status was acceptable. Other eligibility requirements included adequate blood cell counts, except for patients with a positive bone marrow biopsy, and liver function values less than three times normal. All patients gave informed consent.

Treatment consisted of *m*-AMSA administered by iv infusion every 3 weeks. The initial dose of *m*-AMSA was 120 mg/m^2 given over 30-60 minutes. Patients with extensive prior treatment could start at 60 mg/m^2. The dose was increased by 20 mg/m^2 every 3 weeks until 160 mg/m^2 was reached or myelosuppression encountered. Dose modification was required for myelosuppression, hepatic toxicity, mucositis, or extreme nausea and vomiting. Standard supportive care was provided.

TABLE 1.—Patient characteristics and response data

Characteristic	% of patients
Total No. of patients entered: 104	
No. of eligible patients with follow-up data: 89	
Sex	
Male	57
Female	43
Age (yrs)*	
<50	36
50-69	45
70+	19
Performance status	
0	29
1	27
2	28
3-4	15
Time from initial diagnosis (yrs)†	
1	32
1-2	32
3-4	18
5-9	14
10+	4
Prior therapy	
Doxorubicin	91
Radiotherapy	52
Combination chemotherapy	99
Overall response (16 of 89 patients)	18
Complete: 3	
Partial: 13	
Stable disease: 8	
Improved disease: 2	

*Median age, 57 yrs.
†Median, 1.25 yrs.

[1] Received Jan 8, 1986; revised Apr 16, 1986; accepted Apr 24, 1986.
[2] Supported in part by Public Health Service grants CA-31946 and CA-33601 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: Georgetown University, Washington, DC (*P. Schein*, CA-21083); Roswell Park Memorial Institute, Buffalo, NY (*E. S. Henderson*, CA-02599); Wilmington Medical Center, DE (*I. Berkowitz*, CA-37041); Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC (*M. R. Cooper*, CA-03917); Dartmouth Medical School, Hanover, NH (*G. G. Cornwell, III*, CA-04326); Mount Sinai Hospital, New York, NY (*J. F. Holland*, CA-04457); New York Hospital, NY (*R. R. Silver*, CA-07968); State University of New York, Downstate Medical Center College of Medicine, Brooklyn (*S. Rafla*); Walter Reed Army Medical Center, Washington, DC (*R. Weiss*, CA-26806); Finsen Institute, Copenhagen, Denmark (*N. I. Nissen*); Long Island Jewish-Hillside Medical Center, NY (*K. Rai*, CA-11028); Massachusetts General Hospital, Boston (*R. W. Cary*, CA-12449); Columbia University, New York, NY (*R. R. Ellison*, CA-12011); University of California, San Diego (*M. Green*, CA-11789); University of Missouri, Kansas City (*M. Perry*, CA-12046); University of Minnesota, Minneapolis (*B. Peterson*, CA-16450); University of Syracuse, NY (*A. Gottlieb*, CA-21060); Oschner Clinic, New Orleans, LA (*C. Kardinal*, CA-35272); and Dana-Farber Cancer Institute, Boston, MA (*G. Canellos*, CA-32291); and by a grant from the Martell Foundation for Leukemia and Cancer Research.
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TABLE 2.—Response by disease*

	No. of patients	Response		
		Partial + complete	Complete	Partial
Hodgkin's disease	21	4 (19%)	1 (5%)	3 (14%)
Non-Hodgkin's lymphoma	68	12 (18%)	2 (3%)	10 (15%)
Diffuse histiocytic lymphoma	32	6 (19%)	0	6 (19%)
Diffuse lymphocytic lymphoma	0	0	0	0
Poorly differentiated lymphoma	21	4 (19%)	1 (5%)	3 (14%)
Diffuse lymphoma	3	0	0	0
Other nodular and diffuse well-differentiated lymphoma	8	0	0	0
Other non-Hodgkin's lymphomas	4	2	1 (25%)	1 (25%)

* *Duration of response*—18–477 days (median, 176); *survival (all patients)*—44%, 6 mos; 21%, 1 yr; and 11%, 2 yrs; *toxicity*—neutropenia (grade III–IV), 39% (infection 6%); thrombocytopenia, 18%; acute cardiac toxicity, 1%; and allergic reactions, 2%; *dose administered*—full dose, 89%; dose escalation, 30%.

If tumor response or stable disease was documented, treatment was continued until relapse or progressive disease. If progressive disease was seen without toxicity, treatment was continued until two doses of 160 mg/m^2 were given. If the disease progressed and the patient experienced drug toxicity, *m*-AMSA was discontinued after two courses. Responses were defined by published Cancer and Leukemia Group B criteria (5).

RESULTS

One hundred four patients were entered in this study between 1979 and 1981. Fourteen patients were considered inevaluable because of inadequate data (six patients), no treatment (two), inadequate blood cell counts (three), and ineligible diagnoses (three). The overall response rate was 18%, with a 3% complete response rate. Characteristics of the 89 evaluable patients, response rates, and follow-up data are summarized in tables 1 and 2.

Eight patients received only one dose of *m*-AMSA and died during the first month of follow-up. Of these patients, four died of disease, three of cardiopulmonary failure (one patient died 50 minutes following treatment), and one of sepsis.

DISCUSSION

Antitumor activity was demonstrated for *m*-AMSA in these patients with refractory lymphoma but accompanied by moderate toxicity. These results are in accord with several reported trials of treatment of refractory lymphomas with *m*-AMSA (1,2). However, less efficacy was observed than reported by Case (3) and by Bajetta et al (4). Case reported a response rate of 45% in 22 patients with non-Hodgkin's lymphoma, and Bajetta et al reported an overall response rate of 58% (seven responses among 12 patients) in Hodgkin's disease and 30% (14 responses among 46 patients; six complete responses) in non-Hodgkin's lymphoma. The Case study was small (22 patients) and differences in results may be due to patient selection including previous therapy, which was not detailed, and performance status. This discordance with the Bajetta study may be related to drug dose, which was up to 225 mg/m^2 (*m*-AMSA lactate). In that study, however, their response rate did not correlate with dose or difference in drug (*m*-AMSA or *m*-AMSA lactate). No important differences were seen in previous exposure to anthracyclines, previous therapeutic regimens, or performance status to account for the discordant results between this study and that of Bajetta et al.

The results of the current study indicate that *m*-AMSA has limited activity in refractory non-Hodgkin's lymphomas and Hodgkin's disease. Myelosuppression should be anticipated as the dose-limiting toxic effect.

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Phase II Evaluation of Fludarabine in Patients With Metastatic Breast Cancer: A Southeastern Cancer Study Group Trial[1,2]

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Fludarabine is a derivative of vidarabine which, unlike vidarabine, is relatively resistant to deamination by adenosine deaminase and which had previously shown activity in animal models (1). The primary toxic effects observed in phase I trials were granulocytopenia and thrombocytopenia (2,3). Investigators in the Southeastern Cancer Study Group (SEG) undertook an evaluation of the activity of fludarabine in patients with metastatic breast carcinoma from May 1984 through May 1985.

Women with biopsy-proven, incurable metastatic breast carcinoma were eligible if their disease was refractory to no more than one prior chemotherapy regimen (those who had received mitomycin or a nitrosourea were ineligible) and to hormonal therapy if their tumor contained estrogen receptors. A measurable lesion, Karnofsky performance status $\geq 50\%$, written informed consent, recovery from previous myelosuppressive therapy (hemoglobin ≥ 11 g/dl or hematocrit $\geq 32\%$; granulocyte count $\geq 1900/mm^3$; and platelet count $\geq 130{,}000/mm^3$), and a creatinine clearance of at least 70 ml/minute were also required. Cytopenias equivalent to grade I toxicity (3) were allowed if due to myelophthisis (hemoglobin ≥ 9 g/dl; granulocyte count $\geq 1500/mm^3$; and platelet count $\geq 90{,}000/mm^3$). Patients with elevation of hepatic enzymes due to metastasis were eligible. Fludarabine was given at a dose of 25 mg/m^2/day by iv bolus for 5 days every 4 weeks. Doses in the second and succeeding courses were escalated by 20% if no toxicity was observed during the first course of treatment. An adequate trial was defined as two courses of therapy. Toxic effects were graded according to standard SEG criteria (4). A partial response was defined as a reduction of at least 50% in the sum of the products of perpendicular diameters of all lesions. Disease progression was defined as a $\geq 25\%$ increase in the size of existing lesions or the appearance of any new lesion. Stable disease was defined as no significant change for at least 4 weeks, including a $< 50\%$ reduction in size or a $< 25\%$ increase in size.

Fifteen patients were entered; one was not eligible because she had received three previous chemotherapy regimens. Data from the other patients are shown in

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Age (yrs)	
< 50	5
≥ 50	10
Karnofsky performance status (%)	
90–100	5
70–80	8
50–60	2
Menopausal status	
Premenopausal	4
Postmenopausal	11
Dominant site of metastasis	
Hepatic and/or pulmonary	8
Bone and/or soft tissue	6
Hormone receptor status (estrogen or progesterone receptor)	
≥ 10 fmols/g	4
< 10 fmols/g	7
Unknown	4
Response to therapy	
Stable disease	1
Disease progression	13
Ineligible due to 3 previous chemotherapy regimens	1
Toxic effects	
Granulocytopenia (cells/μl)	
1000–2000	3
< 1000	5
Infection while granulocytopenic	1
Thrombocytopenia (cells/μl)	
50,000–130,000	4
< 50,000	1
Hemoglobin	
< 25% decrease	7
≥ 25% decrease	1
Nausea/vomiting (controlled with antiemetics)	4
Abdominal cramps/diarrhea	4
Hepatic* (enzymes ≥ twice normal value)	2
Fever	1
Neurologic†	0

*Elevation of hepatic enzymes was reported in 4 other patients but appears to have been due to progression of metastatic disease.

†Mood changes were reported in 5 patients but on review these seemed to be due to new metastatic disease (2 patients) or situational depression (3 patients).

[1] Received Feb 3, 1986; revised Apr 24, 1986; accepted May 15, 1986.

[2] Supported by Public Health Service grants CA-03227, CA-03376, CA-28138, CA-37577, CA-03117, CA-05634, CA-05641, CA-15584, CA-19657, and CA-24456 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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table 1. No responses were observed. Toxicity was primarily hematologic and gastrointestinal and was generally of moderate severity. No neurological changes due to fludarabine were seen. Fludarabine does not appear to be active against metastatic breast cancer in the doses and schedule used in this study. The drug has also been found to be ineffective when used at a dose of 20–25 mg/m^2/day for 5 days by continuous infusion in a separate study [(5), A. Mittelman, personal communication].

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Phase II Clinical Trial of Acivicin in Advanced Breast Cancer: An Eastern Cooperative Oncology Group Study[1,2]

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Acivicin is an L-glutamine antagonist which kills cells by blocking nucleotide biosynthesis (1). Activity in preclinical models including a breast cancer xenograft was found to be schedule dependent; therefore, we evaluated its activity in advanced breast cancer using a 72-hour infusion schedule (2).

A total of 37 patients with advanced breast cancer resistant to conventional systemic therapy were entered, and of these, 30 were eligible. All patients were required to have been treated with one, but not more than two, prior chemotherapy programs for metastatic disease.

Patients were treated with 60 mg/m^2 of acivicin over 72 hours by constant iv infusion every 21 days. The acivicin dose was escalated to 75 mg/m^2/72 hours in those patients not experiencing toxicity during the first treatment course.

Patient characteristics, toxicity experience, and response to therapy are presented in tables 1 and 2. Of the 25 patients evaluable for response, one had a partial response and the remaining 24 had disease progression. The five unevaluable cases were on study for < 6 weeks: three due to early death, one due to excessive complications, and one due to refusal of treatment. The single responder had multiple pulmonary metastases and pleural disease. Following the first treatment course, lung parenchymal lesions disappeared and the pleural effusion resolved; however, residual abnormalities of the pleura persisted. After 252 days, lung parenchymal lesions and pleural disease progressed; at autopsy the pulmonary lesions were found to be metastatic breast cancer, while the pleural disease was more consistent with a primary mesothelioma. Median survival of the 30 eligible patients was 17 weeks from study entry; median time to treatment failure was 5.6 weeks.

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients (%)*
Age at entry (yrs)	
< 50	9 (30)
50–59	11 (37)
≥ 60	10 (33)
Initial ECOG performance status†	
0, 1 (ambulatory)	22 (73)
2 (nonambulatory, but in bed < 50% of time)	8 (27)
Estrogen receptor	
Positive	13 (43)
Negative	10 (33)
Unknown	7 (23)
Previous chemotherapy	
1 combination	15 (50)
2 combinations	15 (50)
Extent of disease	
1–2 disease sites	16 (53)
3–4 disease sites	14 (47)
Dominant disease sites	
Visceral	18 (60)
Osseous	7 (23)
Soft tissue	5 (17)
Treatment cycles completed	
1 only	13 (43)
2 only	11 (37)
≥ 3	6 (20)
Response (ECOG criteria)‡	
Partial	1 (3)
Disease progression	24 (80)
Not evaluable for response	5 (17)

*Excludes 7 ineligible patients of 37 entered.
†ECOG = Eastern Cooperative Oncology Group.
‡*See* ref 3.

[1] Received Jan 21, 1986; revised May 12, 1986; accepted May 20, 1986.
[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Wisconsin Clinical Cancer Center, Madison (*J. K. V. Willson*, CA-21076); Dana-Farber Cancer Institute, Boston, MA (*M. W. Knuiman*, CA-23318); Medical College of Ohio, Toledo (*R. T. Skeel*, CA-39227); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*J. M. Wolter*, CA-25988); University of Rochester Cancer Center, NY (*K. J. Pandya* and *Y.-C. Chang*, CA-11083); and the University of Pretoria, Republic of South Africa (*G. Falkson*, CA-21692). Other participating institutions include: Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia, PA (CA-18281); Brown University and Roger Williams General Hospital, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Mayo Clinic, Rochester, MN (CA-13650); New York University Medical Center, New York, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); Hospital of the University of Pennsylvania, Philadelphia, PA (CA-15488); and Yale School of Medicine, New Haven, CT (CA-15485).

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TABLE 2.—Toxic effects*

Toxic effect	Mild	Moderate	Severe
Nausea/vomiting	6 (20)	12 (40)	—
Diarrhea	5 (17)	3 (10)	—
Leukopenia	7 (23)	7 (23)	3 (10)
Thrombocytopenia	9 (30)	1 (3)	1 (3)
Anemia	10 (33)	3 (10)	—
Neurologic	7 (23)	9 (30)	3 (10)

*Excludes 7 ineligible patients of 37 entered. Values = No. of patients (%). *See* ref 3 for definition of mild, moderate, and severe.

Neurologic toxicity, granulocytopenia, and nausea and vomiting were the most frequent toxic effects reported. The neurologic toxicity experienced in this trial was similar to that previously reported for acivicin, with lethargy being the most frequent complaint. Two patients developed severe neurologic toxicity which required interruption of the acivicin infusion: one experienced auditory and visual hallucinations, and one experienced nystagmus, unsteady gait, incontinence, and severe depression. Neither patient had evidence of electrolyte imbalance or metastases to the central nervous system at the time. The toxicity resolved following discontinuation of acivicin without residual deficit. A third patient experienced episodic rigors and somnolence for 2 days following completion of the 72-hour infusion. Of the 14 patients receiving more than one course of acivicin, five had the dose escalated to 75 mg/m^2 and all tolerated the escalation without increased toxicity.

The single response among 30 eligible patients indicates that acivicin is not sufficiently active in previously treated patients to recommend further evaluation at this dose and schedule. Similarly, acivicin administered in five daily bolus infusions every 28 days was not active in advanced breast cancer (4).

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Mitoxantrone in Advanced Cervical Carcinoma: A Phase II Study in Patients Not Previously Treated With Chemotherapy[1]

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Mitoxantrone is an anthraquinone derivative with an antitumor efficacy comparable to that of doxorubicin in several tumors (1). It has no proven cardiotoxicity (2), no nephrotoxicity, and a lower incidence of other side effects than doxorubicin (3). For these reasons a phase II study was initiated exploring the efficacy of mitoxantrone in patients with progressive advanced or recurrent carcinoma of the uterine cervix.

MATERIALS AND METHODS

Criteria for entry in the study included histologically proven progressive squamous carcinoma of the cervix with no possibility of curative surgery or additional radiotherapy; measurable and/or evaluable lesions according to World Health Organization criteria; no prior chemotherapy; prior radiotherapy terminated > 3 months previously; performance status of 2 or better (Eastern Cooperative Oncology Group scale); and informed consent according to the Helsinki declaration.

Mitoxantrone was administered at a dose of 14 mg/m^2 iv over a period of 30 minutes once every 3 weeks. Dose escalation was not tried. Hemoglobin values and leukocyte and platelet counts were measured before each course and at the expected nadir 2 weeks later and doses were modified by 10% for leukocyte values at 3–3.9 $\times$ 10^9 cells/L and by 25% for leukocyte values at 2–2.9 $\times$ 10^9 cells/L. Tumor response was assessed by a pelvic examination every sixth week. Cardiac function was monitored by an ECG before each course of treatment and an echocardiogram plus a chest x-ray every 9 weeks. Therapy was continued until evidence of progression of disease was found or a maximum dose of 170 mg/m^2 was reached. An adequate trial was defined as a minimum of two courses.

RESULTS

Eighteen patients with histologically confirmed progressive squamous carcinoma of the cervix were entered. Of these patients 17 were evaluable for response. One patient with a performance status of 3 at entry was excluded. Patient characteristics are shown in table 1.

Upon entry, all patients had normal hepatic and cardiac functions. Hematologic functions were normal, except that two-thirds of the patients had moderate leukocytosis on entry without clinical signs of infection. All patients had normal serum creatinine values, but 12 of the patients (70%) had impaired renal function estimated by Cr-EDTA clearance and seven (40%) had only one functioning kidney due to tumor obstruction.

There were no complete or partial responders.

The major toxic effect observed was moderate leukopenia, with a wbc count nadir 2 weeks after each course of treatment (table 1) and recovery after 1 week. In two patients, 75% doses were given, according to pro-

TABLE 1.—Patient characteristics*

Characteristic	
No. of evaluable patients	17
Median age in yrs (range)	55 (27–73)
Previous therapy	
Surgery only	1
Radiotherapy only	14
Both	2
Hormonal treatment (bromocriptine)	2
Median performance status (range)	1 (0–2)
Measurable disease	
Pelvis	14
Pelvis + distant metastases	3
Median wbc count, $\times$ 10^9 cells/L (range)	
On entry	13.4 (4.0–17.6)
1st course, nadir	4.7 (2.1–14.5)
All courses, nadir	4.3 (0.6–18.0)
Mitoxantrone therapy	
Total No. of courses	79
No. of courses/patient (range)	5 (2–14)
Median cumulative dose, mg (range)	98 (40–280)

*Unless otherwise specified, values = No. of patients.

[1] Received Apr 7, 1986; accepted May 23, 1986.

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tocol. No thrombocytopenia was observed. Other observed toxic effects were moderate alopecia (four patients) and nausea and vomiting (seven). Cardiotoxicity was not observed.

DISCUSSION

The negative reports of mitoxantrone in cervical carcinoma published to date (1,4) have dealt with patients who had previously received at least one chemotherapeutic regimen following radiation therapy. As recently pointed out (5), this implies a risk of excluding a possibly active agent from further evaluation. All patients in this study were previously untreated with cytotoxic drugs, while all but one had received pelvic irradiation to a minimum dose of 50 Gy. No objective response was observed in 17 evaluable patients (response rate, 0%–19.5% at 95% confidence limits). Accordingly, mitoxantrone at the present dosage and schedule has minimal activity, if any, in patients with squamous carcinoma of the cervix.

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Phase II Trial of Esorubicin in Patients With Advanced Colorectal Carcinoma[1]

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Esorubicin is an anthracycline analog of doxorubicin obtained by removing the hydroxyl group from the 4′ position on the amino sugar (1). Preclinical studies demonstrated greater activity of esorubicin compared to doxorubicin, 5-fluorouracil, or carmustine against several human colon tumor xenografts in nude mice (2). Esorubicin had acceptable toxicity in phase I trials, with myelosuppression being the dose-limiting side effect (3,4). We therefore initiated a phase II trial of esorubicin in patients with advanced colorectal cancer.

PATIENTS AND METHODS

All patients were required to have histologic confirmation of colorectal cancer, measurable metastatic disease, wbc count $> 4000/mm^3$, platelet count $> 130,000/mm^3$, serum creatinine < 2.0 mg/100 ml, serum bilirubin < 1.5 mg/100 ml, and Karnofsky performance status (PS) $\geq 50\%$. Patients with evidence of congestive heart failure or significant arrhythmias were excluded.

Thirty-two patients were entered in the study and all are evaluable for response. Patient characteristics are shown in table 1. Esorubicin was administered iv every 3 weeks. The starting dose was 30 mg/m^2 for previously untreated patients with a PS $> 70\%$; all other patients were started at 25 mg/m^2. Previously treated patients were eligible for therapy if they had not received myelosuppressive chemotherapy or radiation therapy within the previous 4 weeks (6 weeks for nitrosoureas). Dose escalation of 5 mg/m^2 was permitted in subsequent courses in the absence of hematologic toxicity. A dose reduction of 5 mg/m^2 occurred in those patients who had a wbc count nadir $\leq 1500/mm^3$ and/or a platelet count nadir $\leq 50,000/mm^3$.

TABLE 1.—Characteristics of 32 patients

Characteristic	No. of patients
Evaluable	32 (100%)
Median age in yrs (range): 58 (38–82)	
Sex	
Male/female	23/9
Median PS (range): 80 (50–100)	
Site of metastatic disease*	
Liver	27
Lung	12
Nodes	2
Peritoneum	2
Prior therapy	
None	12
Chemotherapy	11
Radiation therapy	3
Chemotherapy and radiation therapy	6
No. of cycles	
2-3	23
4-6	9
Toxicity	
Leukopenia	22
Thrombocytopenia	8
Nausea/vomiting	16
Fatigue	13
Alopecia	12
Anorexia	5
Acute venous irritation	5
Mucositis	1
Response	
Partial	0
Stable disease	4
Disease progression	28

*12 patients had ≥ 2 sites of involvement.

RESULTS

No objective responses were seen among 32 evaluable patients, of whom 15 had received no prior chemotherapy. The median number of esorubicin doses was two (range, two to six doses), and the median time to disease progression was 53 days. Twenty-eight patients had unequivocal progression of disease. Stabilization of disease was noted in four patients for 3.5, 4, 4.5, and 5 months.

Esorubicin was well-tolerated. The most significant toxic effect was myelosuppression. Ten patients had wbc count nadirs between 2000 and $3000/mm^3$; nine, between 1000 and $2000/mm^3$; and three, $< 1000/mm^3$.

[1] Received Jan 24, 1986; revised Apr 29, 1986; accepted May 28, 1986.

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Twelve patients had escalation of drug, with one patient receiving up to 40 mg/m^2. Nine patients required dose reduction. Other side effects were mild (grade 1 or 2) and are shown in table 1. No cardiac toxicity was observed in the nine patients who had cardiac follow-up evaluation via radionuclide cineangiography.

DISCUSSION

Despite moderate hematologic toxicity, no objective responses were seen in 32 evaluable patients, of whom 15 were previously untreated, with a 95% confidence interval of 0%–11%. In support of our findings, antitumor activity of esorubicin in a human tumor clonogenic assay demonstrated that only one of 14 colon tumors tested had intermediate sensitivity (5). We conclude that esorubicin has no significant activity in patients with colorectal carcinoma at this dose and schedule.

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Etoposide and Mitoguazone in Refractory or Recurrent Non-Hodgkin's Lymphomas of the Unfavorable Histologic Subtypes[1,2]

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Second-line regimens are needed for the treatment of patients with non-Hodgkin's lymphomas (NHL) of the aggressive histologic subtypes. Individually and in combination with other agents, mitoguazone (MGBG) and etoposide (VP-16) have been reported to have activity in advanced and refractory lymphomas (1-5); hence, a phase II study of these agents was initiated.

MATERIALS AND METHODS

Adults with either stage III or IV NHL of the unfavorable histologic subtypes who had failed first-line therapy were eligible for this study. A creatinine of < 2.0 mg/100 ml, bilirubin of < 2.0 mg/100 ml, performance status of < 4 by Eastern Cooperative Oncology Group (ECOG) criteria, and a life expectancy of > 8 weeks were required.

A 30-minute iv infusion of VP-16 at 75 mg/m^2, with escalation to 100 mg/m^2, and then 125 mg/m^2, if tolerated, was administered on Days 1, 2, and 3 of each 21-day cycle. MGBG at 400 mg/m^2, with escalation to 500 mg/m^2, and then 600 mg/m^2, if tolerated, was infused iv on Days 1 and 8, initially over 30 minutes and later over 1 hour after hypotension with loss of consciousness occurred with the shorter infusion. Standard ECOG response criteria were employed.

RESULTS

The pretherapy characteristics of the 24 patients entered and the results and toxicity of treatment are summarized in table 1. Hematologic toxicity was the most common complication of therapy, resulting in two deaths from sepsis during neutropenia.

The only patient to achieve a clinical complete remission entered the study at the time of first relapse. There were two brief partial responses.

DISCUSSION

Although MGBG and VP-16 have been shown to have activity in NHL (1-5), they were only minimally effective in the doses and schedule utilized in this investigation. This may be in part attributable to the fact that most patients had been heavily pretreated and had far-

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients (%)
Total	24
Median age in yrs (range): 64 (44-78)	
Histologic subtype	
Diffuse, poorly differentiated lymphocytic	6 (25)
Diffuse, mixed cell	4 (17)
Diffuse, histiocytic	14 (58)
Disease status	
First relapse	4 (17)
Refractory	20 (83)
Prior therapy	
Radiation	11 (46)
Chemotherapy	24 (100)
No. of regimens	
1	9 (37)
2	10 (42)
3	5 (21)
Ann Arbor stage	
III	5 (21)
IV	19 (79)
B symptoms	17 (71)
Performance status (ECOG)	
0-1	8 (33)
2-3	16 (67)
Extranodal disease	18 (75)
Bone marrow	11 (46)
Pleural effusions or ascites	7 (29)
Liver or lung	5 (21)
Skin	1 (4)
Median No. of cycles (range): 3 (1-8)	
Major toxic effects	
Leukopenia (wbc count < 2000 cells/mm^3)	8 (33)
Death from sepsis during neutropenia	2 (8)
Thrombocytopenia (platelet count $< 25{,}000$ cells/mm^3)	5 (21)
Intractable nausea and vomiting	2 (8)
Ileus	1 (4)
Wasting syndrome	2 (8)
Paresthesias	2 (8)
Response	
Complete	1*
Partial	2†

*Duration of 7 mos.
†Durations of 2 and 4 mos.

[1] Received Feb 13, 1986; revised May 19, 1986; accepted June 3, 1986.
[2] Supported in part by Public Health Service grant CA-15145 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and the Veterans Administration (Medical Research).
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advanced disease at the time of study entry. Future trials of new therapies for NHL should limit enrollment to patients who have failed a single treatment regimen.

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Phase II Trial of Dichloromethotrexate in Epidermoid Carcinoma of the Esophagus[1,2]

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Dichloromethotrexate (DCM), a 3,5-dichloro derivative of methotrexate (MTX), is a potent inhibitor of dihydrofolate reductase and may be a better substrate for the active transport system than MTX (1). Previous trials have noted activity in cancers of the head and neck and the bladder (2,3). Since MTX has some antitumor activity in epidermoid carcinoma of the esophagus (4,5), a phase II trial of DCM was conducted in this disease.

Eligibility requirements included: advanced cancer not curable by radiation or surgery, measurable or evaluable disease, Karnofsky performance status $\geq 50\%$ and life expectancy > 6 weeks, wbc count $\geq 4000/mm^3$, platelet count $\geq 150{,}000/mm^3$, BUN ≤ 30 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, and bilirubin < 2.0 mg/100 ml. Signed informed consent was obtained from all patients. DCM was administered as a single iv injection of 400 mg/m^2 each week. The dose was escalated by 100 mg/m^2 each week to a maximum dose of 800 mg/m^2 if mucositis was not excessive, the wbc count was $\geq 3000/mm^3$, and the platelet count was $\geq 150{,}000/mm^3$.

Twenty-seven patients were entered in this study (table 1). Five patients were not considered evaluable; review of the pathology in two patients revealed adenocarcinoma, and three patients received one dose of DCM and refused further therapy. No complete or partial responses were seen. Minor responses were seen in three patients, lasting 1, 2, and 5.5 months. The median number of doses of DCM was four and the median maximal dose achieved was 600 mg/m^2. Sixteen patients were not escalated to the projected maximal dose of 800 mg/mg^2 due to thrombocytopenia (two patients) or progression of disease (14 patients).

The median wbc count nadir was $6000/mm^3$ (range, 1900–14,600) and the median platelet count nadir was $227{,}000/mm^3$ (range, 54,000–686,000). Thrombocytopenia prevented escalation of DCM > 400 mg/m^2 in two of three patients with a prior history of cirrhosis, both of whom had normal liver function prior to therapy. Median serum creatinine was 1.2 mg/100 ml, but four patients had a rise in serum creatinine > 2.0 mg/100 ml unassociated with significant emesis or dehydration. One patient experienced reversible drug-induced nephrogenic diabetes insipidus. Emesis was experienced in six patients and never exceeded grade 2 toxicity. Grade 1 mucositis was seen in seven patients.

TABLE 1.—Characteristics of patients entered on study

Characteristic	No. of patients
Entered	27
Evaluable	22
Median age in yrs (range): 63 (48–79)	
Median performance status (range): 70 (50–80)	
Male:female	19:8
Prior therapy	
None	13
Chemotherapy	4
Radiation therapy	3
Chemotherapy and radiation therapy	7
Response	
Complete	0
Partial	0
Minor	3

DISCUSSION

Single-agent chemotherapy in the treatment of advanced esophageal cancer remains disappointing. MTX has marginal antitumor activity in epidermoid carcinoma of the esophagus, although recent studies involving doses of 200 mg/m^2 have been associated with higher response rates (5). DCM, although active in epidermoid carcinoma of the head and neck (2), failed to demonstrate activity in this disease using this dose and schedule. The lack of a major response in 14 evaluable patients who had not received prior chemotherapy indicates activity of $< 20\%$, with 95% confidence, in this population. Nephrotoxicity was more substantial than expected. However, DCM in combination with cisplatin has not resulted in nephrotoxicity exceeding that of cisplatin

[1] Received Mar 24, 1986; revised May 28, 1986; accepted June 9, 1986.
[2] Supported by contract N01-CM-57732 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3] Solid Tumor Service, Departments of Medicine (*D. Bajorin* and *D. Kelsen*) and Diagnostic Radiology (*R. Heelan*), Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.
[4] We thank Angela Burrano and Roberta Lipperman for their technical assistance.
* *Reprint requests to:* David Kelsen, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

alone (3). The renal toxicity seen in this study may be a result of either direct toxicity from the drug or the poor hydration and alimentation in this patient population.

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Phase II Trial of Acivicin in Advanced Breast Carcinoma: A Cancer and Leukemia Group B Study[1,2,3]

Bruce W. Booth,[4,5] Ann H. Korzun,[6] Raymond B. Weiss,[4,5,*] Rose Ruth Ellison,[7] Daniel Budman,[8] Ali Khojasteh,[9] and William Wood[10,11]

Acivicin is an amino acid antitumor antimetabolite isolated from a fermentation broth of *Streptomyces sviceus* (1,2). Because of structural similarity to glutamine, acivicin is a potent inhibitor of L-asparagine synthetase and other L-glutamine amidotransferases, thus inhibiting pyrimidine, purine, and L-asparagine production. Protein, RNA, and, mainly, DNA synthesis are inhibited (1,2). Acivicin had antitumor activity against L1210 and P388 leukemias as well as human lung and mammary cancer xenografts (1,2). In phase I studies, myelosuppression and/or neurotoxicity were dose-limiting (2). We evaluated the activity of acivicin in advanced breast cancer.

MATERIALS AND METHODS

Twenty-one women with histologically confirmed metastatic adenocarcinoma of the breast were entered in the trial. Eligibility, response, and risk criteria have been previously described (4). Acivicin was given iv daily for 5 days at a dose of 15 mg/m^2/day for good-risk patients or 12 mg/m^2/day for poor-risk patients. Treatment was repeated every 21 days for at least two cycles unless disease progression was rapid.

RESULTS

Of the 21 patients entered, one was never treated and five were ineligible (three had received more than two different prior chemotherapy regimens, one had a performance score of 4, and one had radiotherapy 5 days prior to acivicin). Thus, 15 patients were evaluable. In addition, toxicity but not response could be evaluated in one patient who was lost to follow-up 10 days after therapy. Characteristics and toxic effects are shown in table 1. All patients had been heavily pretreated. The average number of cytotoxic drugs previously used was 4.7 per patient. Five patients were assigned to receive the lower dose. Ten patients were assigned to receive the higher dose, but one of these mistakenly received the lower dose.

There were no responses to acivicin in this study. Three patients temporarily had stable disease. The median time to treatment failure was 1.2 months and the median survival time was 5.1 months, with four patients still alive at time of analysis.

Toxicity was significant (table 1). Drug-induced neutropenia was a contributing factor in the death of one patient, and life-threatening thrombocytopenia was seen in another. Severe toxicity was more frequent in patients receiving the lower drug dose. Mild to moderate neurotoxicity, primarily lethargy, confusion, and depression, was common.

DISCUSSION

Previous antagonists of L-glutamine, although long-studied, have been inactive or too toxic in clinical cancer trials. Unfortunately, both this and a similar phase II trial (3) have failed to show any response to the newer glutamine antagonist, acivicin. Although studies of

[1] Received Feb 10, 1986; revised June 2, 1986; accepted June 23, 1986.

[2] Supported by Public Health Service grants CA-33601, CA-26806, and CA-12011 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: University of Missouri, Columbia (*M. Perry*, CA-12046); West Virginia University, Morgantown (*P. Raich*, CA-28562); Cornell Medical Center, New York, NY (*R. R. Silver*, CA-07968); Rhode Island Hospital, Providence (*L. Leone*, CA-08025); and the McGill Cancer Center, Montreal, Quebec, Canada (*J. L. Hutchison*, CA-31809).

[3] The opinions expressed in this article are those of the authors and do not necessarily reflect those of any Government agency.

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[11] We thank Ms. M. Rice and Ms. Janis Paige for their assistance in the preparation of this report.

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TABLE 1.—Patient characteristics and toxicity

Characteristic	No. of patients
Entered	21
Evaluated	15
Median age in yrs (range): 60 (51–89)	
Menopausal status	
Postmenopausal	15
Natural	12
Surgical	3
Estrogen receptor status	
Negative	6
Positive	5
Unknown	4
Performance score*	
0	5
1	4
2	6
Dominant disease site	
Soft tissue	7
Osseous	1
Visceral	7
Prior therapy	
Hormonal therapy	8
Radiotherapy	8
Chemotherapy	15
1 regimen	3
2 regimens	12
Toxicity	
Nausea, vomiting†	4
Leukopenia, nadir (cells/mm^3)	
3000–3999	4
2000–2999	2
1000–1999	2
Thrombocytopenia, nadir (cells/mm^3)	
100,000–149,000	2
25,000–74,999	1
< 20,000	1
Neurotoxicity‡	7

*0 = normal; 1 = ambulatory; and 2 = < 50% of time in bed.
†2 mild and 2 severe.
‡3 mild, 3 moderate, and 1 severe.

acivicin in combination with other agents that would be biochemically synergistic might prove effective, acivicin in this dose and schedule had no activity in patients with heavily pretreated advanced breast cancer.

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ANNOUNCEMENTS

Association for Brain Tumor Research Opens Applications for 1987 Fellowships

The Association for Brain Tumor Research, Chicago, IL, is now accepting applications for fellowships from postdoctoral candidates whose intent is to pursue a career in brain tumor research. Preference will be given to MDs who are US citizens or permanent residents of the United States, seeking research training that will enable them to pursue careers as clinical investigators.

AFBTR fellowships offer an award of $18,000 per annum plus a $2000 annual travel allowance for a period of 2 years, with a third-year renewable option. Each year's travel must include one presentation to the AFBTR Board of Directors in Chicago.

All applications will be reviewed by the Association's Medical Advisory Council and awards will be made based on their recommendations.

Criteria for selection will include the quality of the individual applicant; the quality of the program to which s/he is applying; and the research s/he is undertaking.

Applications must be submitted by the sponsoring program director, and the deadline for completed applications, including letters of recommendation, is *January 9, 1987.* Awards will be announced by April 1, 1987, for fellowships to begin July 1, 1987.

Application forms may be obtained from the Association for Brain Tumor Research, Suite 200, 6232 N Pulaski Rd, Chicago, IL 60646, and completed applications should be mailed to the same address.

For further information, contact: Donald Segal, Executive Director (telephone: 312-782-3800).

New and Future Technology in Cancer Treatment

Cleveland, Ohio, December 5-6, 1986

The Cleveland Clinic Foundation is sponsoring a continuing medical education program entitled "New and Future Technology in Cancer Treatment." The meeting is approved for Category I accreditation and will be held in Bunts Auditorium at the Cleveland Clinic.

For further information, contact: Center for CME, The Cleveland Clinic Educational Foundation, 9500 Euclid Ave, Rm TT3-301, Cleveland, OH 44106 (telephone: 800-762-8173).

International Symposium on Prostatic Carcinoma

Sanremo, Italy, January 22-24, 1987

For further information, contact: F. Boccardo, MD, Scientific and Organizing Secretariat, 1st International Symposium on Advances in Urologic Oncology, Divisione di Oncologia Clinica, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10, 16132 Genova, Italy (telephone: 010-317, 843).

Oncology Practice 1987: A Perspective

Snowmass, Colorado, February 1-7, 1987

For further information, contact: Stephen E. Jones, MD, Sammons Cancer Center #4800, Baylor University Medical Center, 3500 Gaston Ave, Dallas, TX 75246.

35th Annual Meeting of the Radiation Research Society

Atlanta, Georgia, February 22-26, 1987

For further information, contact: Radiation Research Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3153).

American Radium Society Sixty-Ninth Annual Meeting

London, England, April 6-10, 1987

The Portman Inter-Continental in London will be the site of the Sixty-Ninth Annual Meeting of the American Radium Society.

For further information, contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

International Conference on Reproduction and Human Cancer

Bethesda, Maryland, May 11-13, 1987

The National Cancer Institute and the National Institute of Child Health and Human Development are sponsoring an International Conference on Reproduction and Human Cancer to be held in Bethesda, MD, May 11-13, 1987, to focus on the effects of cancer and its treatment on reproduction, including possible genetic effects. Poster sessions are planned to accommodate offered papers.

For further information, contact: Drs. John J. Mulvihill (Landow Bldg, Rm 8C41) or Richard J. Sherins (Bldg 10, Rm 10N234), National Institutes of Health, Bethesda, MD 20892; or call Conference Manager, Ms. Mary E. Clark (telephone: 301-589-6760).

Critical Issues in Tumor Microcirculation, Angiogenesis and Metastases

Pittsburgh, Pennsylvania, June 8-12, 1987

A 5-day course entitled "Critical Issues in Tumor Microcirculation, Angiogenesis and Metastases: Biological Significance and Clinical Relevance" will be given at Carnegie Mellon University, Pittsburgh, PA. Topics covered will include the following: tumor blood flow and its modification; tumor metabolism in vivo; tumor pH; vascular permeability; interstitial transport; uptake of drugs and macromolecules including monoclonal antibodies and biological response modifiers; pharmacokinetics, thermal characteristics of tumors; temperature distributions during normo- and hyper-thermia; tumor angiogenesis and antiangiogenesis; preneoplasia; metastatic process; significance of tumor microcirculation in tumor growth; angiogenesis and metastasis; relevance of tumor microcirculation in cancer detection and treatment.

Laboratory session will include demonstrations of tissue isolated tumor preparation; quantitative microscopy of tumor microcirculation; and measurement of cell deformability. The course meets the criteria for 35 CME credits.

For further information, contact: R. Hilda Diamond, Associate Director, Biomedical Engineering Program, Carnegie Mellon University, Pittsburgh, PA 15213-3890 (telephone: 412-268-2521).

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NIH Publication No. 86-22

October 1986